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A REVERSED ACTION OF THE VAGUS ON THE MAMMALIAN HEART.

By H. H. DALE, P. P. LAIDLAW AND C. T. SYMONS[1].

(*From the Wellcome Physiological Research Laboratories.*)

Introductory. A cardio-accelerator action of the mammalian vagus has frequently been described, and in several instances has been regarded as indicating the presence in the vagus itself of true accelerator fibres. The earlier observations, such as those of Rutherford[(2)] (who, indeed, explained the phenomenon otherwise), of Boehm[(3)], or of Schiff[(3)], and the later experiments of Arloing[(4)], whether the accelerator action was revealed by the aid of drugs such as atropine or curare, or by degenerative section of the nerve, are alike open to explanation by the existence in the vagus of recurrent sympathetic fibres, arising from the stellate or the inferior cervical ganglion, joining the vagus by one of the communicating branches which unite it with the anterior limbs of the annulus, and looping back to their distribution in the cardiac branches. We have ourselves in one case, which will be mentioned later in more detail, obtained a result by means of degenerative section similar to those of Arloing, and susceptible of the same explanation. The phenomenon which is our main concern in this paper is of a different kind. It can be reproduced with regularity under given conditions, and we shall give evidence that it has no connexion with the true sympathetic system. Whether it is due to the presence in the vagus of fibres which are normally accelerator in function is still open to question.

[1] The phenomenon with which this paper deals was first observed accidentally by Dale and Symons in 1907 and partly investigated: after an interval the investigation was resumed and brought to its present stage by Dale and Laidlaw. For preliminary note see This *Journal*, XXXIX. *Proc. Phys. Soc.* p. xiii. 1909.

The production of the reversed action.

For the production of the phenomenon in its most characteristic form certain conditions are necessary. The animal which exhibits it best is the cat. In one experiment on a ferret we obtained it without difficulty. In the dog it is seen, if at all, in a very weak form. In the rabbit we have never succeeded in obtaining the effect. The choice of an anæsthetic is not a matter of indifference. In our earlier experiments we used urethane, with varying success as regards the production of the phenomenon. Latterly we have found that the use of paraldehyde, which has little depressant action on the heart, enabled us to obtain uniformly successful results. The cats were anæsthetised in the first place with chloroform and ether: under these a tracheal cannula was inserted, and 1·5 c.c. of paraldehyde was then given by a stomach tube, the œsophagus being tied as a precaution against regurgitation. The administration of ether was gradually reduced as the effect of the paraldehyde became established. In the later stages of the experiment ether was usually unnecessary, since deep anæsthesia was maintained by the paraldehyde alone.

Blood-pressure was recorded by a mercury manometer connected with the left carotid artery. For most purposes the manometer movements were used as a record of the heart-beats, but in a few experiments the volume changes of the auricles and ventricles were separately observed by the use of the double cardiometer described by Lehndorff[8], small Brodie's bellows being used as recording instruments.

Either vagus produces the phenomenon equally well, and stimulation may be made with equally good result at any point from the ganglion of the trunk to the separation of the cardiac branches. The right vagus was usually chosen, simply as a matter of convenience, the artery cannula being in the left carotid. In a cat anæsthetised and prepared as above a preliminary stimulation of the vagus produced well-marked inhibition of the heart with the secondary coil at distances from the primary varying from 25—15 cm. in different animals. To produce the reversal phenomenon one of several drugs may be used. We observed it first with tropine, injected intravenously in doses of 50—150 mgm. Latterly we have used nicotine in intravenous doses of 1—2 mgm., with equally good results. Hordenine methiodide, the

action of which, as shown by one of us, is in most respects closely similar to that of nicotine, may be substituted in doses of 10—20 mgm., but the effect is more evanescent. Curare will also produce the phenomenon, though in less striking form. We will for convenience take as a type for description an experiment in which nicotine is used.

After the preliminary stimulation, for observation of the normal action of the unpoisoned vagus, 1 mgm. of nicotine is injected intravenously. The rise of blood-pressure resulting is allowed to pass off, when it will be found that the heart-beat is somewhat slower than before the nicotine was given. The vagus is again stimulated. If no inhibition is produced during stimulation the secondary coil is moved about 2 cm. nearer to the primary and the stimulus repeated. If inhibition now occurs during the stimulus another dose of 1 mgm. of nicotine is injected. Stimulation of the vagus will now produce either no inhibition during the stimulus, or a mere preliminary slowing for one or two beats, succeeded by a return to the original rate or, in some cases, a slight acceleration beyond it. When the stimulus ceases the rate gradually becomes slower. If no further stimulus is given this after-slowing, or delayed inhibition, gradually passes off and the original rate is regained. If, however, the stimulus be renewed, during the period of slow beating succeeding the first stimulus, the beat, with scarcely noticeable latent period, is rapidly accelerated up to or beyond the initial rate. The faster rate is maintained as long as stimulation is continued, and a further period of more marked slowing appears when the stimulation again ceases. If this process be repeated, the stimulation being in each case renewed before the slowing succeeding the previous

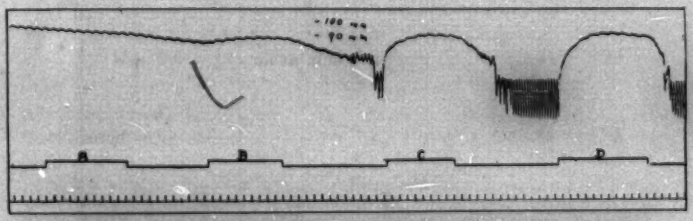

Fig. 1. [In this and other reproductions of kymographic tracings the lines of tracing, from above downwards, unless otherwise mentioned, are carotid blood-pressure (mercury manometer), signal key, and clock marking intervals of 2 seconds.]

Cat. Paraldehyde. Effect of four successive stimulations of the vagus after 1 mgm. of nicotine. At A and B coil at 20 cm.; at C and D coil at 18 cm.

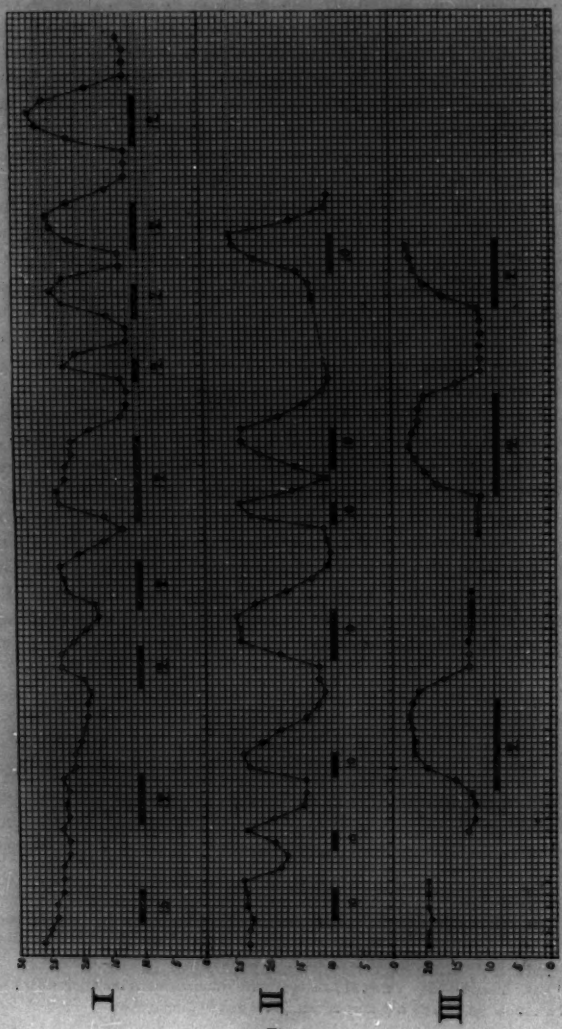

Fig. 2.

Fig. 2. Chart plotted from kymograph records of three experiments. Along the abscissæ time-intervals of 6 seconds are shown: the ordinates indicate the number of heart-beats in such intervals. The shaded rectangular areas indicate periods of vagus stimulation, the figures below them showing coil-distance in centimetres.

I. Development of the reversal phenomenon after 2 mgm. of nicotine.

II. ,, ,, ,, ,, ,, 100 mgm. of tropine.

III. Two longer periods of vagus stimulation from another experiment, in which complete reversal had been developed after 100 mgm. of tropine.

period of stimulation has passed off, the effect gradually becomes intensified, till after four or five such periods of stimulation the appearance is produced of marked inhibition *between* the stimuli, acceleration *during* the stimuli—the picture being almost the exact reverse of that produced by successive stimulations of the vagus in the normal animal.

Fig. 1 illustrates the onset and development of the phenomenon after 1 mgm. of nicotine. The chart (Fig. 2) was constructed from kymograph records by plotting as ordinates the number of heart-beats in consecutive periods of six seconds. It shows the development of the phenomenon after 100 mgm. of tropine (I) and after 2 mgm. of nicotine (II). The effects of two more prolonged periods of stimulation of the vagus, after the phenomenon was fully developed, are plotted in curve (III).

It has already been stated that the two vagi will give the effect equally well. They may be stimulated alternately with an effect not differing from that produced by repeated stimulation of one only. For example, stimulation of the left vagus, during the period of slow beating

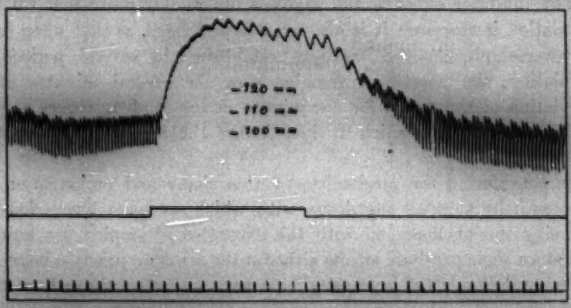

Fig. 3. Cat. Paraldehyde. Stimulation of the vagus after 60 mgm. of tropine. Coil 10 cm. Complete reversal.

following stimulation of the right, produces an acceleration quite similar to that produced by renewed stimulation of the right. (See Fig. 8.) It will be convenient to divide the phenomenon for consideration into two phases, and to examine separately the cause of the after-slowing, and that of the re-acceleration on renewal of the stimulation.

I. *The slowing of the heart-beat after cessation of the stimulus.*

We think that there can be little doubt that this phase of the effect is due to delayed inhibitor action of the vagus. If atropine is injected during a period of slowing the beat is promptly accelerated to a rate equal to or greater than that which obtained before the first stimulation period of the series. Apart from its time-relations this phase has all the characters of vagus-inhibition. By the use of Lehndorff's double cardiometer, which records the volume-change of auricles and ventricles separately, we found that the retardation affected the former concurrently with the latter (Fig. 4). The same observation can be made by simply watching the pulse in the exposed jugular vein. All the chambers of the heart are, therefore, affected as in normal vagus inhibition. All degrees of this delayed and prolonged inhibition were observed in different experiments: in one the inhibition was complete, the beat of the ventricle ceasing suddenly when stimulation of the vagus was stopped, and reappearing only after several seconds (Fig. 5) unless stimulation of the vagus was meanwhile renewed, in which case the beat began again promptly and rapidly rose to the rate obtaining during the previous period of stimulation.

The inhibitor effect of the vagus is not merely postponed till the stimulation is stopped: it is also greatly prolonged, so that when once the reversal phenomenon has been established by several periods of stimulation, the heart-beat remains slow for several minutes after stimulation of the vagus for one minute or less, and only very slowly regains the rhythm which it had before the first stimulus of the series.

In considering the mechanism of this delay and prolongation, it is noteworthy that all the drugs with which we have succeeded in producing the phenomenon, with the exception of tropine, are known to produce their paralytic effects either at the synaptic junction between the pre-ganglionic and post-ganglionic neurone or on the cell of the latter. The fact that tropine produces, in this instance, an effect similar to that of nicotine, curare and hordenine methiodide, may

possibly indicate that it, too, has a weak paralytic action of the nicotine type: it has been shown by several observers to have no true atropine action. It seems reasonable to suppose, therefore, that one phase of the phenomenon is due to delay and prolongation of the normal inhibitor effect of the vagus by partial obstruction to the passage of

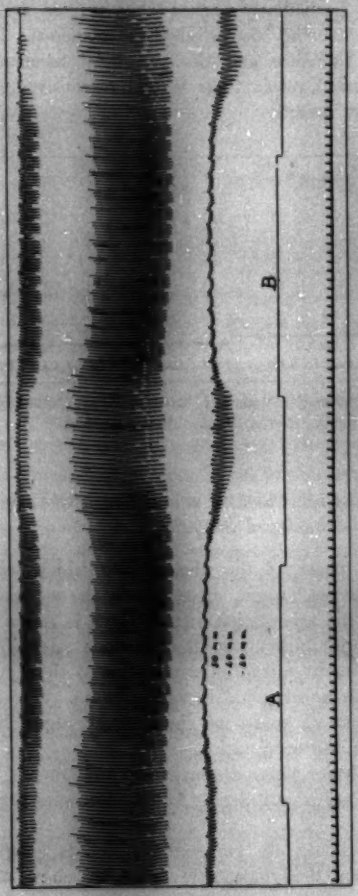

Fig. 4. Cat. Paraldehyde. Lehndorff's double cardiometer. Lines of tracing from above downwards show auricle volume, ventricle volume, carotid blood-pressure, signal key, time in seconds. 2 mgm. of nicotine. At A and B stimulation of right vagus: coil at 15 cm.

impulses across the synaptic junction. This is in accordance with the accepted fact that changes in the time-relations of nerve-impulses occur normally only at synaptic junctions. It is further supported by the observation that, when the reversal phenomenon has been fully developed, pilocarpine still produces its normal cardio-inhibitor effect, as does also a further and larger injection of nicotine. This inhibitor effect of nicotine was particularly marked when tropine was the drug primarily used in producing the reversal phenomenon. The post-ganglionic neurones, therefore, and the peripheral mechanism with which they are connected, retain their normal responsiveness to such stimuli as affect them directly.

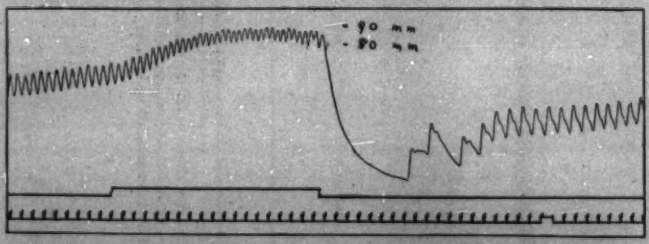

Fig. 5. Cat. Urethane. 150 mgm. of tropine. Complete delayed inhibition succeeding stimulation of the left vagus. Coil at 12 cm. Time in seconds.

II. *Acceleration of the heart-beat by stimulation of the vagus during delayed inhibition.*

Turning to that phase of the phenomenon which leads us to describe it as a "reversed" vagus effect, namely the re-acceleration of the heart-beat when the stimulation of the vagus is renewed during a period of delayed inhibition, the first obvious possibility to be considered is that of mere spread of current on to the sympathetic accelerators. This can be excluded in several ways.

(1) Stimulation at all points of the cervical vagus is equally effective in producing the acceleration.

(2) The use of tripolar electrodes makes no difference.

(3) Pinching the nerve peripherally to the electrodes prevents the acceleration.

(4) In one experiment we exposed the stellate ganglion with its rami, the annulus, and the sympathetic cardiac branches. A stimulus,

which, applied to the sympathetic accelerators at any point, during a period of delayed inhibition, produced no effect or a barely perceptible acceleration, caused marked acceleration when applied to the vagus of the same side. (Fig. 6.)

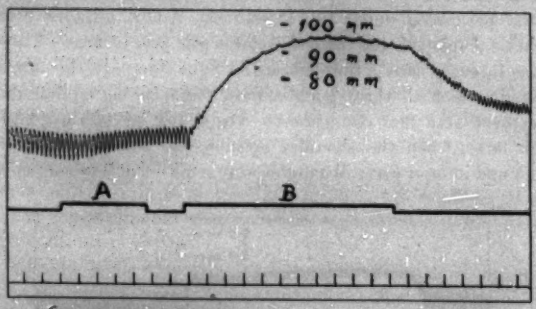

Fig. 6. From same experiment as Fig. 1.

A. Stimulation of right sympathetic accelerators, coil at 16 cm.
B. Stimulation of right vagus, coil at 16 cm.

Current escape is not, therefore, a possible explanation.

A second possible explanation of the abnormal accelerator effect is that it is due to sympathetic post-ganglionic fibres running in the vagus trunk, their effect being normally overpowered, when the vagus is stimulated, by the intrinsic inhibitor fibres. The close connexion of the vagus with the annulus in the cat, especially on the right side (cf. figure by Boehm[6]), and the fact that strands of non-medullated fibres can be traced running between them when they are teased apart, lend support to the suggestion that fibres from the stellate or inferior cervical ganglion may run up for some distance in the vagus and loop back to their distribution. We have already used the possibility that such fibres exist to explain such results as those of Rutherford, Schiff, Boehm and Arloing. The fact that some of these observers obtained more marked accelerator effects with the right than the left vagus is in favour of this explanation of their results. Most of the reasons which exclude current escape may be urged, however, against the attribution of our phenomenon to the presence of such fibres. But for the complete exclusion of this possibility, and of the further possibility that accelerator fibres may arise in the superior cervical

ganglion and join the vagus therefrom[1], degeneration experiments are necessary. Such were therefore undertaken. In a series of experiments the superior cervical ganglion, the inferior cervical ganglion, and the stellate ganglion were removed in separate cats, and in addition all three were removed from each of two cats. The operation was in all cases performed under full anæsthesia, A.C.E. mixture being the anæsthetic employed, and with full antiseptic precautions. The operation was in every case performed on the right side. In the case of the two cats in which all three ganglia were removed the stellate ganglion was removed at a first operation by Anderson's[(7)] method, and about a week later, when the shoulder incision had healed completely, the superior and inferior cervical ganglia were removed at a second operation.

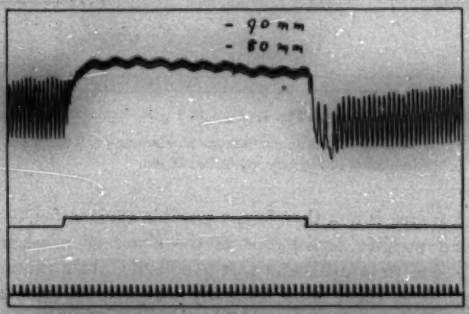

Fig. 7. Cat. Paraldehyde. Superior and inferior (middle) cervical and stellate ganglia removed by operation 12 days previously. 1 mgm. nicotine. Stimulation of the right vagus: coil at 15 cm.

Healing occurred in all cases by first intention. Degeneration was allowed to proceed for varying periods up to 31 days, this latter period being allowed in one of the experiments with extirpation of all three ganglia. At the end of the period of degeneration the animal was anæsthetised in the usual manner with urethane or paraldehyde and the right vagus tested for the production of the phenomenon of reversed action. In all cases it was produced in quite a normal manner. Fig. 7 shows the reversed action of the right vagus in a cat from which all three ganglia were extirpated on the right side, degeneration being allowed

[1] Langley (Schäfer's *Text-book of Physiology*, Vol. II. p. 663) suggests the presence of such fibres in the vagus in explanation of Rutherford and Schiff's results.

for 12 days subsequent to the second operation. It is clear, therefore, that fibres of the true sympathetic system can play no part in the production of the effect.

The accelerator effect being thus undoubtedly due to fibres belonging to the vagus itself, we have a further choice of possibilities to consider.

In the first place, it is conceivable that an actual reversal of function takes place under the influence of the drug, fibres which are normally inhibitor becoming accelerator under the abnormal conditions of the experiments. On such a view the primary effect would be the delay and prolongation of inhibition. During this period of delayed inhibition the post-ganglionic inhibitor neurone must be in a state of mild continuous activity. It is at least conceivable that impulses reaching it from the pre-ganglionic fibre under these conditions might interfere with the continuous excitation, the result being escape of the heart from inhibition while the stimulus lasts. There are, however, several objections to such a view. The effect of the first stimulation after administration of the paralytic drug is frequently a perceptible acceleration while the stimulus lasts, though this is naturally not so marked by contrast as the acceleration produced by subsequent stimulation during periods of delayed inhibition. Secondly, the acceleration is not a mere removal of the delayed inhibition: when the phenomenon has been well established by successive periods of stimulation, the acceleration frequently carries the rate above that which obtained subsequent to the administration of the drug, but prior to the first period of stimulation. Thirdly, a condition of not quite complete reversal is frequently met in which the first effect of the onset of each period of stimulation is to cause a further inhibition, lasting for a second or two, and followed by acceleration which then lasts till the stimulation is stopped. None of these features of the phenomenon can easily be reconciled with the hypothesis of interference.

The other, and on the whole the more obvious explanation, is that the vagus contains, in addition to its normally predominant inhibitor fibres, others which produce acceleration of the heart-beat. The function of the paralyzing drug in this case would be discriminating, the accelerator action being revealed by depression of the inhibitor effect which normally masks it.

If we accept the view that the vagus contains cardio-accelerator fibres, we could suppose these to be (*a*) pre-ganglionic autonomic fibres

like the inhibitors; (*b*) post-ganglionic autonomic fibres, having their cell-station in the jugular ganglion or the ganglion of the trunk; (*c*) fibres which are normally sensory in function, but capable, on artificial excitation, of producing acceleration by antidromic action, the effect being analogous to the vaso-dilatation caused by stimulating dorsal spinal roots (Stricker[9], Bayliss[8]). We have unfortunately not found it possible to discriminate experimentally between these possibilities. With none of the various methods, which have been used in other directions to reveal admixtures of fibres having different

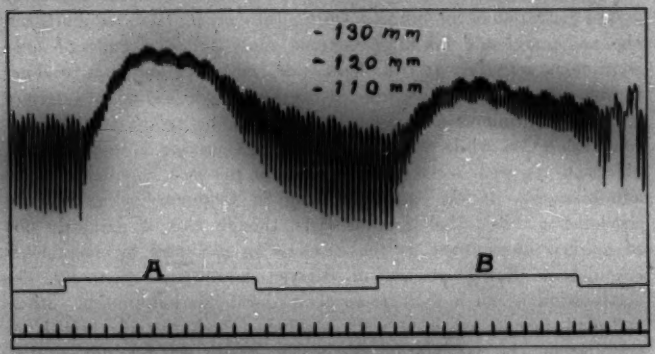

Fig. 8. Cat. Paraldehyde. Right vagus cut 2 days previously.
A. Stimulation of right vagus, coil at 12 cm.
B. „ left „ „ „
Time in seconds.

functions and origins, have we succeeded in producing acceleration with the vagus, in the absence of the paralyzing drug. Slow rhythmical stimulation by induction shocks, repeated make-and-break of a constant current, mechanical excitation, have, in our experience, produced either no effect or inhibition. Nor could we separate the two effects by fractional degeneration. In anæsthetised cats one vagus was cut with aseptic precautions, and degeneration allowed for varying periods, at the end of which the animals were again anæsthetised with paraldehyde and the production of the reversal phenomenon investigated. We found that the accelerator action, under the conditions producing reversed action of the other vagus, persisted in the divided nerve as long as the normal inhibitor action and no longer. After two

days' degeneration the complete phenomenon was obtained, delayed inhibition and re-acceleration during the succeeding stimulus being produced in the characteristic manner. (Fig. 8.) In another case, in which degeneration had proceeded for four days, stimulation of the degenerated nerve produced neither inhibition nor acceleration, before or after the injection of a small dose of nicotine: nor did stimulation of the divided nerve produce any trace of re-acceleration when applied during a period of delayed inhibition, succeeding stimulation of the sound nerve of the opposite side (Fig. 9).

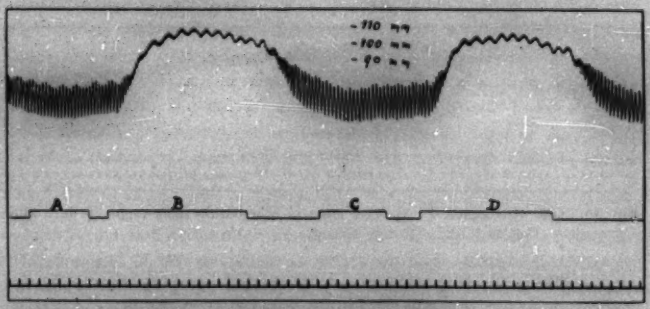

Fig. 9. Cat. Paraldehyde. Right vagus cut 4 days previously.

A. Stimulation of right vagus, coil at 8 cm.
B. ,, left ,, ,, 11 cm.
C. ,, right ,, ,, 5 cm.
D. ,, left ,, ,, 11 cm.

An exceptional result, to which reference has already been made, may conveniently be mentioned here. In one cat only the right vagus, 19 days after section, produced on stimulation a marked acceleration of the heart-beat (Fig. 10). Unfortunately the nerve was not cut high in the neck; but even with the comparatively short peripheral portion available it was easy to satisfy ourselves that the acceleration effect increased as the electrodes were shifted towards the heart. The acceleration, moreover, was only obtained from any portion of the nerve with comparatively strong stimulating shocks. (Secondary coil at 10 to 8 cm. from the primary.) We attribute this result, therefore, like those of Schiff and Arloing, to the presence of recurrent fibres from the sympathetic ganglia. No sound medullated fibres could be

detected microscopically in the nerve after fixation in osmic acid and careful teasing.

On one other point we obtained decisive evidence. If the accelerator fibres are post-ganglionic or sensory their cell-station must be in the jugular ganglion. The vagus was cut in a cat under A.C.E. and with aseptic precautions, just above the ganglion of the trunk. After degeneration for ten days it was tested as usual, the cat being under urethane. No inhibitor or accelerator effect was obtained. This almost suffices for the exclusion of an antidromic effect as the cause of the acceleration: for even if the possibility be admitted that a few

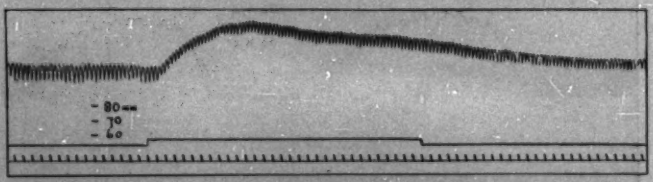

Fig. 10. Cat. Urethane. Exceptional effect of stimulating right vagus 19 days after section. Coil at 8 cm. Time in seconds.

sensory fibres from the heart may have their trophic centre in the jugular ganglion[1], it seems most improbable that these alone should be capable of conveying antidromic impulses causing acceleration, the fibres connected with cells in the ganglion of the trunk being ineffective in this direction. The possibility remains that the jugular ganglion may contain cells of the autonomic type, giving origin to post-ganglionic accelerator fibres. This could only be excluded by intracranial section of the vagus roots, with subsequent degeneration. We have been unable to devise a method for performing this operation in the cat.

Keeping this in view as a possibility, it seems to us, nevertheless, that the balance of probability is in favour of the presence in the vagus of pre-ganglionic fibres, stimulation of which causes acceleration of the heart. The chief difficulty in the way of this conception is that it postulates a discrimination by the paralyzing drug between cells or synapses concerned with inhibition and acceleration respectively. Such discrimination is no new conception in the case of more peripherally

[1] According to Langley all the sensory fibres found in the vagus below the ganglion of the trunk probably have their trophic centre in that ganglion. (This *Journal*, xxvii. p. 280. 1901.)

acting alkaloids. Langley[30] showed, for example, that atropine abolished the motor before the inhibitor effect of the vagus on the rabbit's cardiac sphincter. An abolition of motor effects of sympathetic nerves and of adrenine by ergotoxine, without noticeable impairment of their inhibitor effects, has been described by Dale[31]. Some of the effects produced by small doses of ergotoxine are, indeed, strongly reminiscent of the reversal of the vagus effect on the heart. Stimulation of the splanchnic nerves, for example, after a small dose of ergotoxine, frequently causes a small initial rise of arterial blood-pressure, giving way to a fall which persists while the stimulation continues, to be succeeded by a further prolonged rise when the stimulation ceases. These effects have been generally accepted as indicating a discrimination by the paralyzing alkaloid between motor and inhibitor elements in the peripheral receptive mechanism. Such discrimination in the case of ganglion-cells has, to our knowledge, not been described hitherto, but it seems to us to present no greater inherent difficulty of conception than the analogous process at the periphery.

The view that the accelerator effect is due to the presence of normally masked accelerator fibres also renders easier the explanation of that phase of the phenomenon which we have described as delayed inhibition. It is a common effect of a paralyzing drug, in doses short of those necessary for complete annulment of an effect, to produce a reduction of intensity associated with increased persistence. If we suppose that the effect of the inhibitor neurones is thus altered, it is easy to understand why it should be overcome, during the application of the stimulus, by an accelerator effect which is not similarly depressed and prolonged. When the stimulation ceases the accelerator effect rapidly passes off and allows the prolonged, weak inhibitor effect to emerge.

The fact that the phenomenon has been observed only in such typically carnivorous species as the cat and the ferret is in favour of the theory of selective paralysis as opposed to that of reversal of function. It was in these types that the most striking instances were observed of mixed motor and inhibitor function in sympathetic nerves, as revealed by ergotoxine. It was in the cat, again, that Brodie and Dixon[32] observed a mixed motor and inhibitor action of the vagus on the muscles of the bronchioles. On the whole, therefore, while realising that the somewhat complicated conditions, needed for the production of the phenomenon in its most striking form, make caution desirable in

drawing conclusions, we are of opinion that the balance of probability is in favour of the view which regards the vagus supply to the cat's heart as of mixed function, though predominantly inhibitor under normal conditions. Such an admixture of accelerator fibres would help to explain the readiness with which the cat's heart escapes from vagus inhibition.

NOTE ON AN ANALOGOUS PHENOMENON OBSERVED ON THE CAT'S PUPIL.

We have in several experiments observed a modified response of the cat's pupil and nictitating membrane to stimulation of the cervical sympathetic, which we regard as analogous to the reversed vagus effect on the heart, described above. The cats have usually been anæsthetised with paraldehyde, in the manner already described, but this does not seem to be a point of such importance in this instance, as the phenomenon can be observed in cats under chloroform or ether.

The cervical sympathetic nerve on either side is first exposed and faradised in the usual manner, the weakest stimulus which will just produce maximal dilatation of the pupil being thus determined. Nicotine in 0·1 % solution in saline is then painted on the superior cervical ganglion and the cervical sympathetic stimulated from time to time, the coil being approximated as the effect becomes weaker. If the dilator effect is not abolished by 0·1 % nicotine, stronger solutions up to 0·5 % are employed. Ultimately the pupil does not respond to any strength of stimulus. The animal is now left for a time and the stimulation again applied, and repeated at intervals. In some cases nothing occurs until ultimately a weak normal dilator effect reappears. In most cases, however, after a short time, a curious delayed effect is observed. A slight and rapid dilatation may occur at the onset of the stimulus, but this quickly passes off and the pupil remains in its semi-contracted condition while the stimulus continues. As soon as it ceases slow dilatation of the pupil and retraction of the nictitating membrane appear, and the dilated condition persists for some seconds. If now, during this period of delayed dilatation, the stimulus is renewed, a slight but quite distinct constriction of the pupil and advance of the nictitating membrane occurs and persists while the stimulation lasts, again giving way to delayed dilatation and retraction when the stimulation stops. Quite similar results were obtained by painting 0·2 % hordenine methiodide on the superior cervical ganglion. When once the

phenomenon has been produced it is not easily abolished by further painting of the ganglion with nicotine.

We have made no analysis of the effect, except to exclude the participation of possible pre-ganglion fibres running through the superior cervical ganglion and making connexion with cells of the ciliary ganglion. Injection of 1% nicotine into the orbit intensifies the effect if it alters it at all.

The effect is a small one and needs close watching for its observation, but, in a favourable case, the edge of the iris may be seen to move 1—2 mm., while the movement of the nictitating membrane, though less constant, is sometimes the most prominent feature in the effect.

It seems to us almost certain that this phenomenon and the heart-effect with which this paper is principally concerned must be explained along the same lines.

A reversal of the action of the pelvic nerve on the cat's bladder, produced by curare, was demonstrated by Prof. Langley[13] to the Physiological Society on July 9th. In this case, again, the order of events, viz. a preliminary contraction at the onset of the stimulation, followed by relaxation while the stimulation continued, and a further, much larger and more persistent contraction following the cessation of the stimulus, is strongly suggestive of analogy with the reversed vagus effect on the heart. Langley regards his observation as indicating the presence in the cat's pelvic nerve of an admixture of inhibitor fibres for the bladder.

Summary.

A reversal of the effect of the vagus on the heart of the cat is described, by which acceleration of the rhythm during electrical stimulation of the nerve is produced with inhibition following the cessation of the stimulus. It is uncertain whether the phenomenon is due to actual reversal of the function of normally inhibitor fibres, or to the presence in the vagus of normally masked accelerator fibres. The latter seems the more probable view.

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CHEMICAL STRUCTURE AND SYMPATHOMIMETIC ACTION OF AMINES.

By G. BARGER and H. H. DALE.

(*From the Wellcome Physiological Research Laboratories.*)

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Introductory. The elucidation of the structure of the active principle of the supra-renal medulla by the work of Abel[1], v. Fürth[2], Takamine[3], Aldrich[4], Pauly[5], and Jowett[6], and its synthesis by Stolz[7] and by Dakin[8], almost simultaneously, led to the physiological investigation of substances nearly related to it in chemical structure. Loewi and Meyer[9], working with Stolz's preparations, investigated a number of ketones of the general formula $(OH)_2C_6H_3 . CO . CH_2NR_1R_2$ and the corresponding secondary alcohols $(OH)_2C_6H_3 . CHOH . CH_2NR_1R_2$, obtained by reduction of the ketones, among these reduction products

[1] *Johns Hopkins Hosp. Bull.* VIII. p. 151. 1897. *Zeitschr. f. physiol. Chem.* XXVIII. p. 318. 1899.

[2] *Zeitschr. f. Physiol. Chem.* XXIX. p. 105. 1900. *Hofmeister's Beitr.* I. p. 248. 1901.

[3] *Amer. Journ. of Pharmacy*, LXXIII. p. 523. 1901.

[4] *Journ. Amer. Chem. Soc.* XXVII. p. 1074. 1905.

[5] *Ber. d. deutsch. chem. Ges.* XXXVI. p. 2944. 1903 and XXXVII. p. 1388. 1904.

[6] *Journ. Chem. Soc.* LXXXV. p. 192. 1904.

[7] *Ber. d. deutsch. chem. Ges.* XXXVII. p. 4149. 1904.

[8] *Proc. Roy. Soc.* LXXVI. B. p. 491. 1905.

[9] *Arch. f. exp. Path. u. Pharm.* LIII. p. 213. 1905.

being synthetic dl. adrenine. Dakin examined similar series[1] and found, as Loewi and Meyer had done, that in most cases the reduction of the ketones to the secondary alcohol greatly intensified the adrenine-like action which most of the bases possessed in some degree: but in the case of several of the ketones examined by Dakin, in which the amino-group was substituted by more complicated radicles, no such increase of activity on reduction occurred.

All the active bases examined by these and other observers have been catechol derivatives. Dakin, indeed, concluded that the catechol nucleus was the essentially active group in the adrenine molecule, basing this view on the observation that catechol itself causes a rise of blood-pressure when injected intravenously, while the base methyl-amino-ethanol, $CH_2(OH)CH_2.NHCH_3$, which may be regarded as the detached side-chain of the adrenine molecule, has no such action. The same conception has been adopted by Schultz. Recently, however, we found that certain amines produced by putrefaction, none of them possessing a catechol nucleus, have an action very similar to that of adrenine. On the other hand we shall give evidence that the rise of blood-pressure produced by catechol is due to an action of an entirely different type. It became clear, therefore, that the conception put forward by Dakin, and adopted by others, was inadequate, and it became of interest to trace the variations in the intensity of the specific action through a series of compounds intermediate between the putrefactive animes—in particular *p.* hydroxyphenylethylamine—and adrenine itself. This was the main object of our investigation, but incidentally the changes of physiological activity accompanying other changes of structure have been studied.

G. Barger has throughout been responsible for the chemical side of the investigation, and the new compounds dealt with, except where other sources are acknowledged, have been prepared by him or Mr A. J. Ewins. Only brief indications of the processes of preparation are given here, details being reserved for publication elsewhere. For the physiological experiments H. H. Dale is alone responsible.

Experimental Methods. The action with which we are concerned is the immediate peripheral effect on involuntary muscle and gland cells produced by intravenous injection. This, in the case of adrenine itself, has been shown (Langley[2], Brodie and Dixon[3], Elliott[4]) to

[1] *Proc. Roy. Soc.* LXXVI. B. p. 498. 1905.
[2] This *Journal*, XXVII. p. 237. 1901.
[3] *Ibid.* XXX. p. 476. 1904.
[4] *Ibid.* XXXII. p. 401. 1905.

simulate with considerable precision the effects of stimulating nerves of the true sympathetic system, being limited to muscle-fibres and gland-cells innervated by this system and producing in them responses which are, for the most part, in the same direction, whether of augmentation or inhibition, as those which the sympathetic nerve-impulses produce. We are dealing with a range of compounds which thus simulate the effects of sympathetic nerves not only with varying intensity but with varying precision. In some cases the points of chief interest in the action of a compound are those in which it differs from the action of adrenine. A term at once wider and more descriptive than "adrenine-like" seems needed to indicate the type of action common to these bases. We propose to call it "sympathomimetic," a term which indicates the relation of the action to innervation by the sympathetic system, without involving any theoretical preconception as to the meaning of that relation or the precise mechanism of the action.

It is clear that the production of a rise of arterial blood-pressure, though a convenient quantitative index of an action referred to this type on other grounds, does not in itself afford adequate ground for such reference. Some or all of the other effects of sympathetic nerves must be observed, and any considerable reproduction of the effects of autonomic nerves other than sympathetic must be excluded, before the action can properly be classified as sympathomimetic. Dilatation of the pupil and contraction of the plain muscle in the orbit, a flow of saliva and tears not readily abolished by atropine, inhibition of the tone and rhythm of the muscular walls of the mammalian intestine and of the cat's urinary bladder, are familiar and easily observed sympathetic effects. Particularly valuable as an index of this type of action is the response of the cat's uterus. This organ is innervated by sympathetic nerves only, and its response to them and to adrenine changes with its functional condition, being in the main motor during pregnancy and inhibitor in its absence. Exceptions to this rule are found occasionally, but it is easy to obtain a cat's uterus of which the activity is simply inhibited by adrenine—the virgin organ is invariably so in our experience—and it can conveniently be treated as an isolated organ, suspended in warm, oxygenated Ringer's solution. Under these conditions the peripherally placed ganglion-cells rapidly lose their responsiveness, so that nicotine and substances which act like it cause contraction of the uterus as an isolated organ, even though they inhibit it, by stimulating sympathetic ganglia, when they are injected into the circulation of the

living animal. We believe that the association of a pressor action, due to arterial constriction and cardio-acceleration, when the substance is injected into the whole animal, with inhibition of the tone and rhythm of the isolated non-pregnant uterus of the cat, is almost sufficient evidence to warrant the attribution to a substance of an action of the sympathomimetic type. We have, however, in all cases confirmed the diagnosis by observing several of the other sympathetic effects above mentioned. In the case of homologous series and closely related compounds we have usually performed this analytical identification of the action completely in the case of one typical member only, being content in the case of others to observe that the action was of the same general type. It must be understood, however, unless the contrary is specifically stated, that the action of any substance spoken of as sympathomimetic has been proved to be so.

As a general quantitative index of the activity of a substance, of which the type of action has been identified as sympathomimetic, we have adopted the effect on arterial blood-pressure. This has obvious advantages in the ease with which it can be observed and measured, and its adoption makes our results to some extent comparable with those of other observers who have used it for this purpose. We shall show, however, that this method affords only a broad and rough indication of the order of activity of the different bases. In the case of closely related bases the relative values obtained by different observers differ somewhat widely. The animals used, the mode of anæsthesia employed, and probably less obvious differences of technique, as well as individual variations of response in animals of the same species, all affect the results. We shall show, further, that the indications of relative activities obtained by the blood-pressure method by no means hold good for the action on other organs innervated by sympathetic nerves. The effect on the blood-pressure is, indeed, so complex that the determination of the relative efficiency of some closely related bases in its production has not much more than a conventional value, until the results are analysed in the light of observations on other sympathomimetic effects. For the comparison of pressor activities we have used decerebrate cats almost exclusively. When other animals were used, or the brain not destroyed, the fact is stated and the anæsthetic mentioned. In most of our experiments the spinal cord was cut at the level of the axis vertebra, the neural arch of the vertebra being removed. Up to this point complete anæsthesia was maintained by ether and chloroform. The brain was then

completely destroyed, bleeding being stopped by modelling clay and by plugging the foramen magnum with a cork. Artificial respiration was then applied. Recently in several experiments the animals have been decapitated under anæsthesia according to the method described by Sherrington[1]. By either method a preparation is obtained which for hours gives a low steady blood-pressure, free from the irregularities due to variations in activity of the respiratory or vaso-motor centre, and which is therefore particularly well suited to the comparison of the effects of pressor substance. In the case of bases most nearly related to adrenine a quantitative comparison of activities can be made with considerable accuracy, since such bases will give practically identical effects with a succession of equal injections. As described by Elliott[2], for estimating the relative strengths of solutions of adrenine, and by Cushny[3], and by Schultz[4] for comparing the activities of different bases, the activities can be expressed as the reciprocals of the doses required to produce equal submaximal rises of pressure, care being taken to make the injections equal in volume and uniform in rate. We made injections always by the femoral vein and recorded the pressure in the carotid artery by the ordinary mercurial manometer. The recording drum was moved back by hand between injections so as to cause overlapping of the curves and facilitate comparison.

As the structure of the amines examined becomes increasingly remote from that of adrenine the accuracy of the comparison becomes continually less. For example, successive equal doses of an amine of the primary fatty series usually produce rapidly diminishing effects. In such a case we have been content to arrange the members of the series in order of activity, testing each in comparison with the next lower and higher in the series. To guard against error due to diminishing response to successive injections we have always given a series of alternating doses, *B* being judged more active than *A* only if, in a series of equal injections, *ABA* or *BAB*, *B* always produces a rise of pressure higher than that produced by the equal dose of *A*. By equal injections, in such a case, must be understood equal volumes of equimolecular solutions, the volumes being so chosen as to give submaximal rises of pressure.

For experiments in isolated organs, suspended in warm oxygenated Ringer's solution, we used an apparatus, which we shall describe in detail in another connexion, which enables the Ringer's solution to be

[1] This *Journal*, xxxviii. p. 375. 1909.
[2] This *Journal*, xxxvii. p. 130. 1908.
[3] *loc. cit.*
[4] *Hygienic Laboratory Bulletin*, No. 55. Washington. 1909.

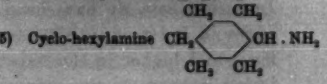
changed without disturbing the continuity of the record. The action of a series of amines could thus be tested on a single isolated organ, such as the horn of a cat's uterus, or the retractor penis of a dog or goat.

A. Aliphatic amines.

Some of our results with this series were outlined in a preliminary note[1]. One of them, isoamylamine, was identified by Barger and Walpole[2] as a pressor constituent of putrid meat: it is probably the "urohypertensine" of Abelous and Bardier[3]: and Barger and Dale[4] found traces of it in ergot. Its action has been described in detail by Dale and Dixon[5]. It is only necessary here to recall that it was shown to have an action definitely of the sympathomimetic type, which, however, differed from that of adrenine, not only in being relatively weak, but in reproducing the motor more powerfully than the inhibitor effects of true sympathetic nerves and in being less strictly limited to structures innervated by that system.

The following members of this series have been tested:—

Primary amines.

(1) Methylamine $CH_3.NH_2$.
(2) Ethylamine $C_2H_5.NH_2$.
(3) *iso.* Propylamine $(CH_3)_2.CH.NH_2$.
(4) *n.* Propylamine $C_3H_7.CH_2.NH_2$.
(5) *iso.* Butylamine $(CH_3)_2.CH.CH_2.NH_2$.
(6) *n.* Butylamine $C_2H_5.CH_2.CH_2.NH_2$.
(7) *iso.* Amylamine $(CH_3)_2.CH.CH_2.CH_2.NH_2$.
(8) *n.* Amylamine $C_3H_7.CH_2.CH_2.CH_2.NH_2$.
(9) *n.* Hexylamine $C_5H_{13}.NH_2$.
(10) *n.* Heptylamine $C_7H_{15}NH_2$.
(11) *n.* Octylamine $C_8H_{17}NH_2$.
(12) *n.* Nonylamine $C_9H_{19}NH_2$.
(13) *n.* Endekylamine $C_{11}H_{23}NH_2$.
(14) *n.* Tridekylamine $C_{13}H_{27}NH_2$.
(15) Cyclo-hexylamine 

Secondary, tertiary amines etc.

(16) Diethylamine $(C_2H_5)_2NH$.
(17) Methylisoamylamine $C_5H_{11}.NH.CH_3$.
(18) Di-isoamylamine $(C_5H_{11})_2.NH$.
(19) Trimethylamine $(CH_3)_3N$.
(20) Tetraethylammonium iodide $(C_2H_5)_4NI$.

Diamine.

(21) Pentamethylene-diamine (cadaverine) $NH_2.C_5H_{10}.NH_2$.

[1] This *Journal*, xxxviii. p. xxii. (*Proc. Phys. Soc.*) 1909.
[2] This *Journal*, xxxviii. p. 343. 1909. [3] *Journ. de Physiol.* p. 84. 1909.
[4] This *Journal*, xxxviii. p. lxxvii. (*Proc. Phys. Soc.*) 1909.
[5] This *Journal*, xxxix. p. 25. 1909.

Most of the above substances were obtained from well-known commercial sources: where necessary they were purified by recrystallising their salts. No. (11) was prepared from *n.* octyliodide and ammonia; Nos. (12)—(14) by Jefferies' method from the amide of the next higher acid; No. (15) by electrolytic reduction of cyclo-hexanone-oxime.

All were tested in $\frac{N}{10}$ solutions as hydrochlorides. The most active members of the group are found among the primary amines. In the doses which were used—1 to 2 c.c. of the $\frac{N}{10}$ solution—the activity did not extend in any significant degree to members lower in the series than *n.* butylamine, which in activity comes immediately below *iso.* amylamine, the type-member, for us, of the series. *Iso.* butylamine had practically no pressor effect in the dosage indicated, though doubtless, if injected in larger volume or stronger solution, it, and even lower members of the series, could be shown to possess a perceptible pressor effect. Under such conditions mere volume-effects complicate the record and we only mention the probability of such activity in order to avoid the suggestion that a new characteristic suddenly appears in the series.

The relative weakness of the *iso.* as compared with the normal bases is shown by the amylamines as well as the butylamines. *Iso.* amylamine

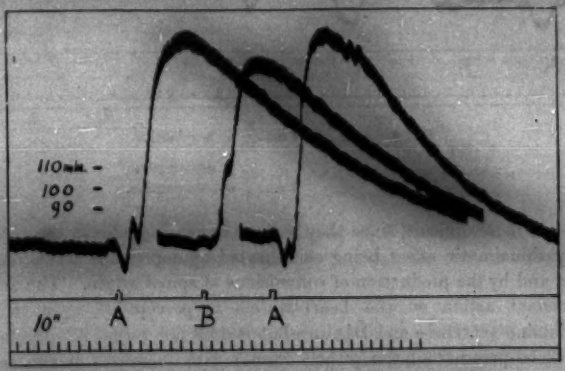


Fig. 1. [Figs. 1 to 4, 6, 8 to 10, 14 and 15 show carotid blood-pressure. Injections intravenous. Bases as hydrochlorides.]

At *A*, *A*, 1 c.c. $\frac{N}{10}$ *n.* amylamine.

B, ,, ,, *iso.* amylamine.

is several times as active as *n.* butylamine: *n.* amylamine is again distinctly, though not very greatly, more active than *iso.* amylamine (Fig. 1). The *iso.* amylamine in question is the base $(CH_3)_2CH_2CH_2CH_2NH_2$, which occurs as a putrefaction product of leucine. The other isomeric amylamines were not available for examination: they would probably have been less active, since, in this series, the activity seems to increase with the length of the straight carbon-chain up to six carbon atoms.

Of the bases above amylamine only the normal (straight-chain) representatives were available. Normal hexylamine is the most active of the normal series. Normal heptylamine is slightly, but distinctly less active (Fig. 2). With the higher members of the series comparison

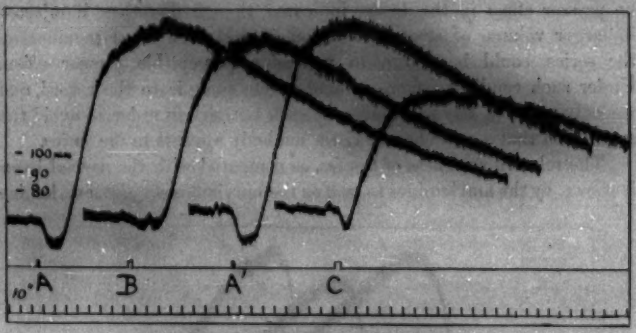


Fig. 2. At *A*, *A'*, 1 c.c. $\frac{N}{10}$ *n.* hexylamine.
B, ,, ,, *n.* heptylamine.
C, 2 c.c. ,, *iso.* amylamine.

becomes more difficult, since they become increasingly toxic, the specific sympathomimetic effect being complicated by depressant action on the heart and by the production of convulsions of spinal origin. The direct, depressant action on the heart-muscle is perceptible even in *iso.* amylamine (cf. Dale and Dixon), but it does not seriously complicate the blood-pressure of the pithed animal with members of the series lower than octylamine. One dose of these higher amines greatly depresses the effect of succeeding doses, so that injections in the order *ABA* are no longer useful for comparison. Octylamine is definitely less active than heptylamine, but beyond that comparison

is impossible, since whichever of the higher bases is injected first produces the bigger effect. It may simply be stated that the pressor action is still distinct in tridekylamine, though the sympathomimetic nature of the pressor effect of these higher bases is merely a probable inference from analogy. Pentadekylamine was prepared, but its hydrochloride is almost insoluble in water and could not be tested. Cyclo-hexylamine (hexahydro-aniline) has a pressor action quantitatively very similar to that of normal hexylamine, though somewhat slower and more prolonged. Sufficient of the base was not available, however, for a detailed examination of its action, and the possibility is open that this is not of the sympathomimetic type.

Of the secondary amines diethylamine was found to be inactive, as might be expected from the negligible activity of *iso.* butylamine. The only other secondary amines examined were those with the *iso.* amyl radicle. Methyl-*iso.* amylamine possesses the action, but is considerably weaker than *iso.* amylamine, being roughly one-half as active. Di-*iso.* amylamine has very little of the action.

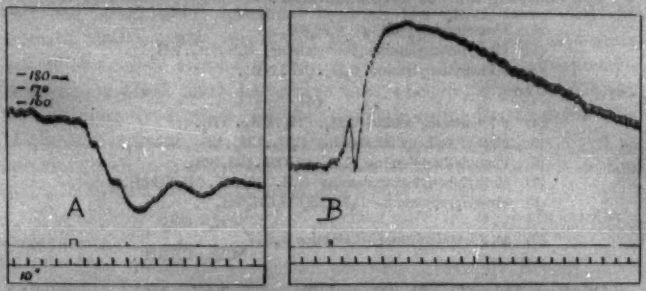


Fig. 3. At *A*, 1 c.c. $\frac{N}{10}$ cadaverine ($NH_2 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . NH_2$).
B, " " " *n.* amylamine ($CH_3 . CH_2 . CH_2 . CH_2 . CH_2 . NH_2$).

Trimethylamine was examined chiefly because a pressor action has been attributed to it by Abelous and Bardier[1]. They were working with a rather higher scale of dosage (1 centigram per kilogram in the dog, as compared with 6—12 milligrams for a cat of 2—4 kilograms, as employed by us) and with an animal with intact vaso-motor centre and normal respiration. Abelous and Bardier find that trimethylamine

[1] *C. R. Soc. de Biol.* LXVI. p. 347. 1909.

is only $\frac{1}{100}$ as active as urohypertensine, which they regard as identical with the base obtained by them from putrid meat, and identified by Barger and Walpole as *iso.* amylamine. We should ignore an action $\frac{1}{100}$ as powerful as that of *iso.* amylamine, and from our point of view trimethylamine is inactive. Of quarternary aliphatic ammonium bases we examined only tetra-ethylammonium iodide. The toxicity of this base is well known, but we found it inactive in the direction under investigation.

Of diamines only pentamethylene-diamine (cadaverine) was tested, which may be regarded as normal amylamine, with one hydrogen atom of the terminal methyl group substituted by a second amino-radicle. Fig. 3 shows that the action is thereby entirely changed, the pressor action of amylamine being replaced, in cadaverine, by a depressor action, the nature of which has not been further analysed.

B. Aromatic amines without a phenolic hydroxyl.

The following amines were examined :—

(1) Phenylamine (aniline) $C_6H_5NH_2$.
(2) Phenylmethylamine (benzylamine) $C_6H_5CH_2.NH_2$.
(3) α-Phenylethylamine $C_6H_5.\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}.NH_2$.
(4) β-Phenylethylamine $C_6H_5.CH_2.CH_2.NH_2$.
(5) Methyl β-phenylethylamine $C_6H_5.CH_2.CH_2.NHCH_3$.
(6) Phenylethanolamine $C_6H_5.CHOH.CH_2NH_2$.
(7) Methylphenylethanolamine $C_6H_5.CHOH.CH_2NHCH_3$.
(8) Phenylpropylamine $C_6H_5CH_2CH_2CH_2NH_2$
(9) *ac.* β-tetrahydronaphthylamine $C_6H_4\begin{cases}CH_2—CH_2\\ \quad\quad\quad|\\ CH_2—CHNH_2\end{cases}$.

Of these Nos. (1), (2), (4) and (9) were obtained from commercial sources. Nos. (3) and (8) were prepared by reduction of the oximes of acetophenone and cinnamic aldehyde respectively, No. (5) was obtained by reducing the product of condensing phenacetaldehyde with methylamine: Nos. (6) and (7) resulted from the action of ammonia and methylamine respectively on styrene chlorohydrin.

Our starting-point in this series was No. (4), β. phenylethylamine, which was identified in putrid meat as a factor in the pressor action. Its activity is distinctly greater than that of the most active (hexylamine) of the aliphatic amines. The action was not so completely investigated as that of *iso.* amylamine and that of *p.* hydroxyphenylethylamine, but

sufficient evidence was obtained to warrant the conclusion that it is of the sympathomimetic type. In addition to the rise of blood-pressure it produces the characteristic dilatation of the pupil, relaxation of the urinary bladder (in the cat), and inhibition of the tone and rhythm of the virgin cat's uterus. Its activity in all these directions is intermediate between that of the fatty amines and that of the phenolic base *p.* hydroxyphenylethylamine. It may be noted that it stands between them also in respect of approach to adrenine in structure. The carbon-skeleton of β-phenylethylamine is, indeed, identical with that of adrenine.

Taking β-phenylethylamine as our starting-point, we investigated first the effect of varying the length of the side-chain. It was shown that lengthening the carbon chain of the purely aliphatic bases up to a certain point was attended with increase of activity. In the case of the fatty-aromatic base, however, we found that the side-chain of two carbon atoms gave the optimum of activity. Aniline, which has no side-chain, and is therefore a purely aromatic base, had none of the specific action: benzylamine had a mere trace: and α-phenylethylamine, in which, again, only one carbon atom intervenes between the amino-group and the aromatic ring, was also very feebly active. Increasing the side-chain beyond two carbon atoms also resulted in a decline of activity, phenylpropylamine being much less active than phenylethylamine (Fig. 4). The optimum constitution of a fatty-aromatic amine for the production of the sympathomimetic action is, therefore, that which is found in adrenine itself, viz. a benzene ring with a side-chain of two carbon atoms, of which the second bears the amino group.

The modifications necessary to produce adrenine from phenylethylamine are:

(i) The introduction of two phenolic hydroxyls in the 3 : 4 position.

(ii) The introduction of a secondary alcoholic hydroxyl on the first carbon atom of the side-chain.

(iii) Methylation of the amino group.

The effect of introducing phenolic hydroxyls will be dealt with in later sections. Modifications (ii) and (iii), separately and together, produce Nos. (5), (6) and (7) of this series. All these bases are active, but none of them differs noticeably in activity from β-phenylethylamine itself. These modifications, therefore, which, as we shall show later, profoundly alter the activity of the bases possessing phenolic hydroxyls, are practically without effect in the absence of the latter.

The action of β-tetrahydronaphthylamine, which may be regarded as cyclo-hexylamine condensed with a benzene nucleus, has been investigated by Jonescu[1], who showed that in most respects it was sympathomimetic. We have, therefore, been content to compare its

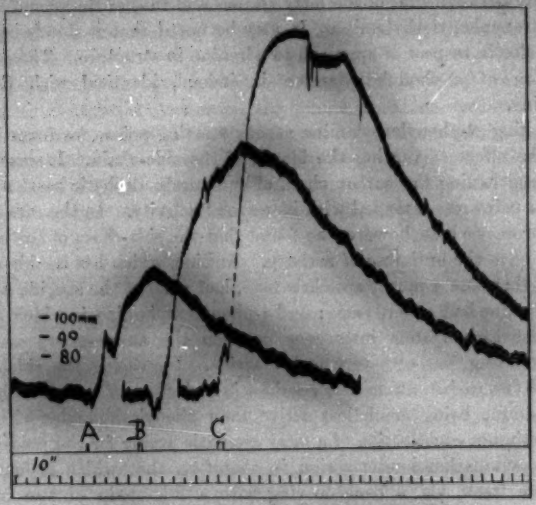


Fig. 4. At *A*, 2 c.c. $\frac{N}{100}$ phenylpropylamine.
B, ,, ,, phenylethylamine.
C, ,, ,, *p*. hydroxyphenylethylamine.

action on the blood-pressure with that of β-phenylethylamine. We found it to be considerably more active than the latter. On the other hand it is distinctly less active than the phenolic base *p*. hydroxyphenylethylamine, which forms our starting-point for the next series.

[1] *Arch. f. exp. Path. u. Pharmakol.* LX. p. 345. 1909.

C. Amines with one phenolic hydroxyl.

The following were tested:—

(1) *p.* hydroxyphenylethylamine

(2) *m.* hydroxyphenylethylamine

(3) *o.* hydroxyphenylethylamine

(4) *m.* methyl *p.* hydroxyphenylethylamine
(*o.* creylethylamine)

(5) *p.* hydroxyphenylethanolamine

(6) *p.* hydroxy *ω.* amino acetophenone

(7) *p.* hydroxyphenylethylmethylamine

(8) *p.* hydroxyphenylethyl-ethylamine

(9) *p.* hydroxyphenylethyldimethylamine
(hordenine)

(10) *p.* hydroxyphenylethyltrimethyl ammonium iodide
(hordenine methiodide)

(11) *iso p.* hydroxyphenylethylamine

(1) was our starting-point in this series, having been identified as a pressor constituent of putrid meat by Barger and Walpole: its action has been described in detail by Dale and Dixon. The base employed for the present investigation was prepared synthetically (cf. Barger, *Journ. Chem. Soc.* xcv. p. 1123. 1909). Nos. (2), (4), (5), (6), (7), (8) and (11) are new substances, and their methods of preparation have been described elsewhere. For Nos. (5) and (6) we are indebted to Mr F. Tutin[1]: for (7) and (8) to Mr G. S. Walpole[2]. A specimen of No. (2) was very kindly sent to us, at our request, by Prof. R. Pschorr

[1] *Journ. Chem. Soc.* xcv. p. 2113. 1909. [2] *Journ. Chem. Soc.* xcvii. p. 941. 1910.

of Berlin. For No. (9), extracted from malt-germs, we are indebted to Mr F. H. Carr. (10) was prepared from (9), and also from (1), by methylation.

Dale and Dixon showed that *β-p.* hydroxyphenylethylamine, No. (1), is at once more powerful (roughly 10 times) and more strictly sympathomimetic in its action than *iso.* amylamine. We have already stated that it is more active than phenylethylamine (cf. Fig. 4). The ratio of the activities of the two seems to vary with the condition of the animal, being in some cases about 1 : 3, in others about 1 : 5. Conditions affecting the sensitiveness of response act unequally in the two cases. The action of the phenolic base appears to be more strictly peripheral, so that the degree of shock to the spinal centres has more influence on the action of phenylethylamine. For our present purpose, however, it is enough to emphasise the fact that the introduction of a phenolic hydroxyl, in the para position, has further increased the activity, already increased, as has been shown, by the presence of the aromatic ring. A phenolic hydroxyl in the meta position is equally effective, the activity of (2) being practically equal to that of (1). In the ortho position it has not this effect, (2) being no more active than phenylethylamine. It should be noted, in this connexion, that the two phenolic hydroxyls of adrenine, and of the allied bases dealt with in the next section, occupy the meta and para positions relatively to the side-chain. The base numbered (11) in this series bears the same relation to (1) as does α-phenylethylamine to *β*-phenylethylamine. Like the former it proved very slightly active. The view was already expressed, in discussing the last series, that the optimum arrangement for the production of sympathomimetic activity in an amine, is the attachment of a benzene ring and the amino-group each to a different carbon atom of a two-linked fatty chain. The presence of a phenolic hydroxyl clearly does not affect the validity of this statement.

Methylation of the ring is known to increase the activity of phenolic compounds in certain directions. For example, cresol is a more powerful germicide than phenol. Methylation of the ring has no such intensifying effect on the sympathomimetic action of phenolic amines. No. (4), which was examined to test this point, was found to be considerably less active than No. (1), being roughly one half as active.

The introduction of a phenolic hydroxyl, forming *p.* hydroxyphenylethylamine from phenylethylamine, which has been shown to cause a notable increase of activity, brings the structure one stage nearer that of adrenine. It might be expected that the association with this

change of substitutions in the side-chain, which assimilate the latter to the adrenine side-chain, would result in a further increase of activity. Such is not the case, however. The necessary modifications are the introduction of the secondary alcoholic hydroxyl, producing No. (5) and the methylation of the amino-group, producing No. (7). No. (5) was prepared for us by Mr Tutin by reduction of No. (6), which was also tested, in view of the known activity of the ketone which yields adrenine on reduction. No. (6) was but feebly active, the activity being of the order of one tenth that of No. (1). Contrary to expectation No. (5) also proved itself less active than No. (1), though considerably more active than No. (6). Qualitatively the activity of (5) does, indeed, make an approach to that of adrenine in so far that the rise of pressure produced is much more rapid and correspondingly more evanescent than that produced by an equipotent injection of (1). This may, with great probability, be attributed to the greatly decreased resistance to oxidation, which the introduction of the alcoholic hydroxyl entails. Quantitatively, however, (5) is greatly inferior to (1) in activity, about 5 mgms. of the former being required to produce a rise of pressure to the same height as that caused by 1 mgm. of the latter.

Methylation of the amino-group of No. (1), which brings its structure nearer to that of adrenine in another direction, failed again to produce the expected increase of activity. The effect of the change is, indeed, surprisingly small. Of three cats, on which the base (1) and (7) were compared in equimolecular solutions, two gave regularly a larger rise of blood-pressure with (1), the other giving, equally regularly, a larger rise with the equivalent dose of (7). In connexion with the catechol bases we shall refer again to these individual differences of reaction. The point which chiefly concerns us now is that the methylation causes no definite increase of activity in this instance. This is true not only of the pressor action, but of the inhibitor action on the non-pregnant cat's uterus, on which (1) and (7) produce closely similar effects. This is the more remarkable in view of the very marked difference produced on the activity of the catechol bases, in this direction, by methylating the amino-group.

No. (8), the corresponding ethylamino-base, was made for us by Mr Walpole, and tested in comparison with (1) and (7) on account of the interesting results obtained with the ethylamino-bases in the catechol series. It proved considerably less active than (1) and (7).

The inhibitor action of these three bases, (1), (7) and (8) was also tested on a virgin cat's uterus. The same order of activity was obtained,

(1) and (7) being practically identical in action, (8) considerably less active (Fig. 5). The dimethylamino-base, No. (9), occurs in nature as the alkaloid hordenine, isolated from malt-germs by Leger[1]. It has been examined physiologically by Camus[2], who found that it produced a rise of blood-pressure, due to peripheral vaso-constriction, accompanied by cardiac inhibition or acceleration, according as the vagi were intact

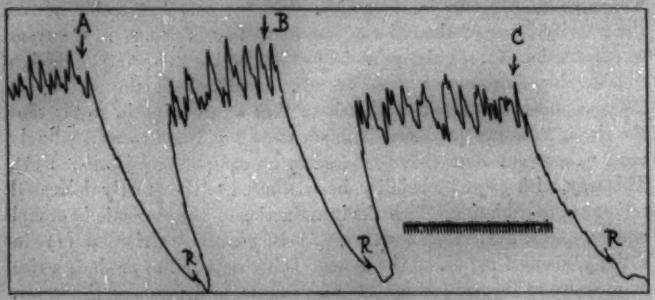


Fig. 5. Isolated uterus of virgin cat in 250 c.c. Ringer's solution. Lever rises with contraction.

At *A*, 2 c.c. $\frac{N}{100}$ *p.* hydroxyphenylethylamine.

B, ,, ,, *p.* hydroxyphenylethylmethylamine.

C, ,, ,, *p.* hydroxyphenylethylethylamine.

At *R*, *R*, *R* the Ringer's solution was changed.

or cut. He observed secretion of saliva, tears, gastric juice and bile, followed by diminished secretion if larger doses were used. Such large doses produced an evanescent paralysis of the vagus and the chorda tympani. We have not investigated the action of these large doses. The pressor action of injections of 2—5 mgms. of hordenine into the pithed cat is slight, but distinct. In this respect hordenine is very much less active than (1) or (7) (Fig. 6). The different effects described by Camus, are not inconsistent with an action mainly sympathomimetic, but more evidence is needed before it could be referred definitely to that type. On account of the relative weakness of the action we have not analysed it further. The relatively very weak action of dimethylaminoacetocatechol, as compared with the corresponding primary and secondary amines, was observed by Loewi and

[1] *Comptes Rendus*, CXLII. p. 108. 1906.

[2] *Arch. intern. de Pharm. et de Thér.* XVI. p. 43. 1906.

Meyer, and presents an interesting analogy. The question whether these tertiary amines really have an action of the same type deserves further investigation.

The action of the quarternary ammonium salt, hordenine methiodide, is of great interest. A large number of such quarternary bases from alkaloids have been examined by various observers, beginning with Fraser and Crum-Brown, and the generalisation that they have a curare action has been weakened by comparatively few exceptions.

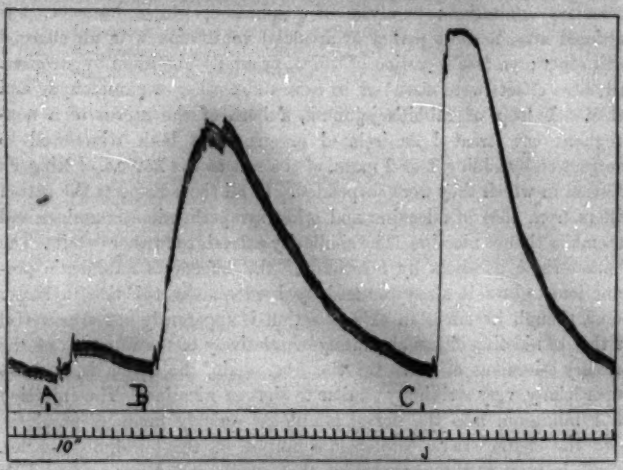


Fig. 6. At *A*, 2 mgms. hordenine.
B, ,, *p*. hydroxyphenylethylamine.
C, ,, hordenine methiodide.

In view of the recent evidence of the physiological antagonism between nicotine and curare (Langley[1]) it was of particular interest, therefore, to find in hordenine methiodide a quarternary ammonium salt of which the action resembles that of nicotine in almost every particular. 1 milligramme of this salt, injected intravenously, causes inhibition of the heart for a few beats, followed by the characteristic rapid rise of blood-pressure and rapid fall (Fig. 6). The effect is indistinguishable from that caused by 0·5 mgm. of nicotine. The effect

[1] *Proc. Roy. Soc.* B, LXXVIII. p. 170. 1906.

on the involuntary eye-musculature varied with the anæsthetic; the typical effect, however, of injecting 1—2 mgms. intravenously into a cat under ether was brief dilatation, followed by constriction of the pupil, narrowing of the palpebral fissure, and forward movement of the nictitating membrane. Painting 0·2 % hordenine methiodide on the superior cervical ganglion caused dilatation of the pupil: injection of a few drops into the orbit through the conjunctiva caused constriction of the pupil. Other effects of intravenous injection were rapid and large contraction of the urinary bladder, quickly giving way to secondary inhibition; paralysis of respiration, which, however, was resumed after a short period of artificial ventilation with air charged with ether; profuse secretion of saliva, promptly abolished by atropine. All these effects were observed in cats under ether or paraldehyde and ether. A loop of rabbit's jejunum, a horn of the uterus of a non-pregnant cat, treated as isolated organs, were both stimulated to contraction by adding 1 or 2 mgm. of the salt to the 250 c.c. of Ringer's solution in which they were suspended. In all these respects the action differs from that of adrenine and other sympathomimetic amines and resembles that of nicotine. The similarity extends to further details. The characteristic paralysis by nicotine of the effects of autonomic pre-ganglionic fibres is also produced by hordenine methiodide in larger doses, though its effect in this direction is apparently not so powerful as that of nicotine either absolutely or relatively to the intensity of the primary stimulant effects. On the frog, again, the action is, at least superficially, very strikingly similar to that of nicotine. The injection of 1 milligram into the dorsal lymph-sac quickly produces paralysis, with the characteristic cataleptic condition of the fore-limb muscles. It will be seen, in the next section, that quarternary bases produced by complete methylation of other sympathomimetic amines have a similar nicotine-like action.

One other observation may be conveniently mentioned here. It will have been noticed that all the substances hitherto dealt with are organic amines. Any modification which destroys the basic properties immediately annuls the activity. Thus the acetyl derivative of *p.* hydroxyphenylethylamine, is devoid of activity. So also is the closely related tyrosine ethyl ester,

in spite of its basic properties.

D. Amines with two phenolic hydroxyls.

Of the amines which we have examined in this group all except one have the phenolic hydroxyls in the ortho-relation to one another (*i.e.* they are catechol derivatives) and in the 3 : 4 position relatively to the side-chain. Among these is, of course, adrenine itself. The catechol bases which we have tested may be classified as follows:—

(*a*) *Derivatives of aceto-catechol* (*ketones*).

(1) Amino-aceto-catechol $(OH)_2C_6H_3 . CO . CH_2NH_2$.
(2) Methylamino-aceto-catechol $(OH)_2C_6H_3 . CO . CH_2NHCH_3$.
(3) Ethylamino-aceto-catechol $(OH)_2C_6H_3 . CO . CH_2NHC_2H_5$.
(4) Propylamino-aceto-catechol $(OH)_2C_6H_3 . CO . CH_2NHC_3H_7$.
(5) Trimethylamino-aceto-catechol chloride $(OH)_2C_6H_3 . CO . CH_2 . N(CH_3)_3Cl$.

(*b*) *Derivatives of ethyl-catechol.*

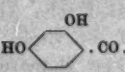
(6) Amino-ethyl-catechol $(OH)_2C_6H_3CH_2CH_2NH_2$.
(7) Methylamino-ethyl-catechol $(OH)_2C_6H_3CH_2CH_2NHCH_3$.
(8) Ethylamino-ethyl-catechol $(OH)_2C_6H_3CH_2CH_2NHC_2H_5$.
(9) Propylamino-ethyl-catechol $(OH)_2C_6H_3CH_2CH_2NHC_3H_7$.
(10) Trimethylamino-ethyl-catechol chloride $(OH)_2C_6H_3CH_2CH_2N(CH_3)_3Cl$.

(*c*) *Derivatives of ethanol-catechol* (*secondary alcohols*).

(11) Amino-ethanol-catechol $(OH)_2C_6H_3CH(OH)CH_2NH_2$.
(12) Methylamino-ethanol-catechol (adrenine) $(OH)_2C_6H_3CH(OH)CH_2NHCH_3$.

Of these catechol bases, those of classes (*a*) and (*c*) have been examined previously by Loewi and Meyer[1], in connexion with the synthetic work of Stolz[1], and by Dakin[1]. Nos. (3), (11), and (12), which are obtainable commercially, have recently been the subject of elaborate physiological comparison by Schultz[1]. Those in class (*b*) are all new. For (7), (8), and (9) we are indebted to Dr F. L. Pyman[2], who has elsewhere described their preparation.

In addition to these catechol bases we have had the opportunity of examining one in which the phenolic hydroxyls are in the 2 : 4 position relatively to the side-chain and therefore in the meta-relation to one another. This substance

(13) 2 : 4 dihydroxy ω aminoacetophenone 

was kindly supplied to us by Mr Tutin.

It will be convenient to begin the consideration of this series by

[1] *loc. cit.* [2] *Journ. Chem. Soc.* xcvii. p. 264. 1910.

examining Dakin's statement that even catechol itself possesses the adrenine action in some degree. His deduction that the catechol nucleus is essential to such action has been sufficiently disproved by the results recorded in the foregoing sections. But since all the substances in which we have been able hitherto to trace the sympathomimetic action are amines, the possession of such an action by a non-basic substance such as catechol would be interesting if substantiated. We confirmed the observation that 5—10 mgms. of catechol, injected intravenously, cause a very distinct rise of blood-pressure. This is true of the pithed cat, as well as of the anæsthetised rabbit, as used by Dakin. The action is, therefore, not wholly dependent on the integrity of the vaso-motor centre. When, however, we looked for the other characteristic features of sympathomimetic action we could observe none of them. No action on the pupil or the urinary bladder of the cat could be observed with intravenous injections. When catechol was added to a bath of Ringer's solution containing an isolated horn of the virgin cat's uterus it caused merely a slight increase in the tone of the organ. Fig. (7) contrasts the effects on such a uterus of catechol and *p.* hydroxyphenylethylamine, showing that the amine without a catechol nucleus reproduces the characteristic sympathetic effect, while catechol itself merely causes increase of tone,

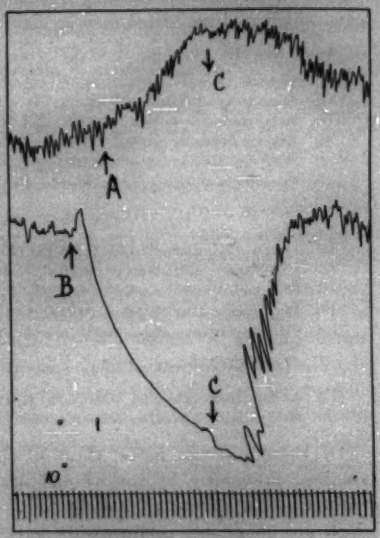


Fig. 7. Isolated uterus of virgin cat.
Upper tracing. Effect of 5 mgms. of catechol at *A*.
Lower tracing. Effect of 2 mgms. of *p.* hydroxyphenylethylamine.
Fresh Ringer at *C*, *C*.

due presumably to direct tonic action on the muscle-fibres. Catechol, so far as our observations go, appears to be a general, not powerful stimulant of plain muscle. When the centres are intact a central action probably contributes to the pressor effect obtained. Its action has no relation to the specific one with which we are concerned, and the description of its action as adrenine-like has no proper justification.

Though catechol itself has not the sympathomimetic action, the bases of this series, which contain a catechol nucleus, possess that action in a much higher degree than those of the series previously examined. By this we do not mean that any one member of this series will be found much more active than any one member, or even always than the corresponding member of the previous series. We shall show that this is not always the case: but the optimum activity in the series of catechol bases is very much greater than the optimum in any of the previous series.

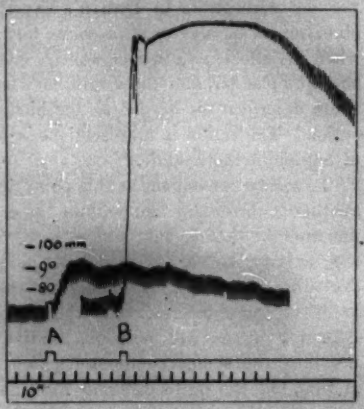


Fig. 8. At *A*, 2 c.c. $\frac{N}{100}$ amino-aceto-resorcinol.
B, „ „ amino-aceto-catechol.

Dakin's experiments showed the importance of the presence of the two phenolic hydroxyls. He found that dimethoxyphenyl bases were inactive, so that the hydroxyl groups must be free. Examination of the base numbered (13) in this series adds another point to our information. This base, which contains a resorcinol instead of a catechol nucleus, is no more active than the corresponding *p.* hydroxyphenyl base, No. (6) of the previous series. The introduction of a second hydroxyl in the 2nd position relatively to the side-chain has, therefore, not increased the activity, whereas if the second hydroxyl occupies the 3rd position, so that a 3:4 dihydroxyphenyl base is produced, the activity is, in this instance, immensely increased (Fig. 8).

Of the catechol bases, those in class (*a*) were examined by Loewi

and Meyer who tested (1), (2) and (3) and by Dakin, who tested (1), (2), (3) and (5). According to Loewi and Meyer the amino-ketone (1) is more active than the methylamino- (2) or ethylamino- (3). Dakin appears to have regarded them as equally active, but found (5) more active than any of them, though he noticed a peculiarity in its action, in that the effect of a large dose lasted no longer than that of a smaller dose. This peculiarity is probably explained by the fact that this base (5), like the other quarternary ammonium bases of this and the previous series, has an action of the nicotine type. This difference was not perceptible in Dakin's experiments, which were limited to observations on the blood-pressure, but immediately becomes apparent when the effects on other organs are studied. The actions on the pupil, the cat's bladder, and the virgin cat's isolated uterus, are all similar to those described for hordenine methiodide and clearly characterise the action. The action in all directions is, however, less powerful than that of hordenine methiodide.

It will be convenient at this point to mention the action of No. (10), another quarternary ammonium base, which again has a typical nicotine-like action, but is more powerful than hordenine methiodide, the activity, as far as could be judged with the small specimen available, approximating closely to that of nicotine itself. It is a point of some interest that the introduction of a second phenolic hydroxyl in the 3rd position, relative to the side-chain, intensifies not only the sympathomimetic action of the primary and secondary amines, but the quite different nicotine-like action of the quarternary ammonium base.

All the other amines of this series have the typical sympathomimetic action in different degrees. In comparing their activities we find it necessary for the first time to give separately the results obtained by different methods. In the case of the bases in previous series the inhibitor activities, when these have been the subject of quantitative comparison, have been found to run parallel to the activities as measured by the blood-pressure. In the case of the catechol bases there is no such parallelism. Nor is the divergence merely due to small differences in the ratios obtained: the accuracy with which the inhibitor effects can be compared is, indeed, not sufficient to permit the assignment of exact ratios. If, however, the bases in one class—*e.g.* the ketones—be arranged in order according to their activity in producing a rise of blood-pressure, or in inhibiting the non-pregnant uterus, the order will not be the same in the two cases.

It will be convenient to describe first the results obtained by the conventional blood-pressure method, and to discuss afterwards the light thrown on these by experiments on other organs.

Effects on the blood-pressure.

Of the bases in class (*a*), Nos. (1), (2), (3) and (4), the most active in nearly all our blood-pressure experiments has been No. (3). No. (1), however, is but little less active, the ratios of activities of (1) to (3), given by the reciprocals of the volumes of $\frac{N}{100}$ solutions necessary to produce equal submaximal rises of pressure, being, in several experiments, 1 : 1·5. In the case of one cat, however, the relation was reversed, the ratio being 1·5 : 1. This exception was so unexpected that we were inclined to attribute it to mere error in labelling the solutions, until we confirmed the result with new solutions, and subsequently found the same reversal of the usual relation of activities of the amino- and ethylamino-bases of class (*b*). There is no room for doubt, therefore, that the result was due to an abnormal sensitiveness of this cat to the amino-bases as compared with its sensitiveness to the ethylamino-bases.

Although the ratio given above, 1 : 1·5, was found to hold generally in our experiments when the comparison was made on the freshly prepared animal, it does not hold good indefinitely even for the same animal. The phenomenon of decline of activity at unequal rates for the different bases is better marked in the case of the bases of classes (*b*) and (*c*), and will be discussed more fully in dealing with them. It is, however, observable with the bases (1) and (3), more of (1) as a rule being necessary to match a given dose of (3) in activity, the longer the animal has been prepared.

The effects of bases (1) and (3), while thus differing quantitatively, are qualitatively very similar. Both cause a rapid rise of blood-pressure succeeded by a fairly prompt fall. When, however, an equal dose of $\frac{N}{100}$ solution of (2) is injected the form of curve obtained is very different. The pressure begins to rise fairly rapidly, but the rise soon becomes slower, and the pressure may remain practically level for a time, or may even fall slightly: then follows a long, slow, secondary rise and fall.

The contrast between the two types of action is illustrated in Fig. (9) and in Fig. (14). It is clearly impossible to make an accurate numerical comparison between actions of such different time-relations

and the statement that the pressor action of (2) is considerably weaker than that of (1) must in this case suffice. The action of (4) is still much weaker. As regards their action on the blood-pressure, then, the order of activity of these four bases is normally (3), (1), (2), (4): exceptionally (1), (3), (2), (4).

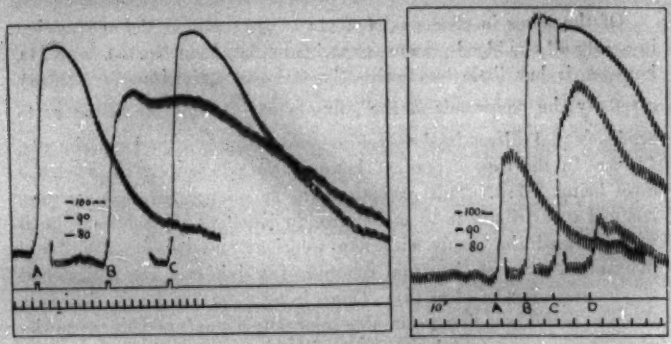


Fig. 9. At *A*, 0·5 c.c. $\frac{N}{100}$ amino-aceto-catechol.
B, „ „ methylamino-aceto-catechol.
C, „ „ ethylamino-aceto-catechol.

Fig. 10. At *A*, 1 c.c. $\frac{N}{200}$ amino-ethyl-catechol.
B, „ „ methylamino-ethyl-catechol.
C, „ „ ethylamino-ethyl-catechol.
D, „ „ propylamino-ethyl-catechol.

In group (*b*) the nicotine-like action of No. (10) has already been described. The other four have the typical sympathomimetic activity in varying degrees. The amino-base of this class (No. 6) is distinctly less active than the amino-ketone, No. (1), the ratio of the activities of (1) to (6), as measured by the blood-pressure effects, being about 1·5 : 1. The methylamino-base of the ethyl-catechol class, No. (7), on the other hand, is not only many times as active as the corresponding ketone, No. (2), but is the most active of all the compounds yet examined, with the exception of those in class (*c*), which includes adrenine itself. The ratio of activities of (6) and (7) is found to vary somewhat widely not only in different animals, but in the same animal at different stages of the experiment. Thus we have determined the ratio as 1 : 5 at the beginning of a long experiment; redetermined

later in the same experiment it has been found 1 : 10. The activity of the ethylamino-base, No. (8), seems to bear a more constant relation to that of the amino-base, the ratio being about 1·5 : 1, as in the corresponding bases of the ketone class (*a*). Here again, however, the ratio increases somewhat as the sensitiveness of the animal declines. The propylamino-base of class (*b*), No. (9), is again much the least active of those examined (Fig. 10).

In class (*c*) only two amines have been available for examination the amino- and methylamino-bases, the lævo-rotary naturally occurring form of the latter being adrenine. Of the amino-base we have had only the *dl.* form, and for purposes of comparison the synthetic *dl.* adrenine has therefore been employed as well as the natural *l.* base. It was first pointed out by Cushny[1] and confirmed by Abderhalden and Müller[2] that the *dl.* is considerably less active than the *l.* form, the ratio according to Cushny's determinations being 1 : 2. At the time of his publication we had already made a similar comparison and had obtained a somewhat different ratio, viz. 6·5 : 10. Recently Schultz[3] has published the results of a very careful comparison, made with a very carefully purified sample of the natural base and gives the ratio as 1 : 1·5. This agrees more closely with our own result, but we do not regard the small discrepancy between his results and our own, on the one hand, and Cushny's on the other, as having any significance. A study of Schultz's protocols shows that he observed, as we also did, some variation in the ratio in different experiments, and the differences in the methods of anæsthesia used by the different observers would be quite sufficient to account for some numerical difference in their results. On the other hand Biberfeld's[4] clearly erroneous statement that the *dl.* is as active as the *l.* base is attributable to his use of the minimal-effective-dose method, with which accurate results are impossible.

The relative activities of the *dl.* adrenine and *l.* adrenine being thus somewhat variable, it was thought better to eliminate one source of uncertainty in making comparison with the amino-base, and for this purpose the synthetic *dl.* adrenine was therefore used. We agree with Schultz that the *dl.* amino-base (No. 11) is usually distinctly more active than the *dl.* methylamino-base (No. 12) on the blood-pressure. Schultz gives the ratio as 1·5 : 1. We determined it, in different

[1] *loc. cit.* [2] *loc. cit.*
[3] *Med. Klinik.* 1906. No. 45.
[4] *Zeitsch. f. physiol. Chem.* LVIII. p. 185. 1908.

experiments, as 1·5 : 1, and 1·25 : 1. Not only, however, did we find that it varied somewhat in different experiments, but even in the same experiment the relation of the activities became altered or even reversed. In a freshly prepared pithed animal the amino-base was always slightly the more active: but if the animal were left for some hours, under artificial respiration with warmed air, and the comparison again made, it was usually found that the methylamino-base was now as distinctly the more active as it had initially been the less active of the two.

We have mentioned now in several instances this variation in the relative responsiveness of the animal to different bases with varying condition. The varying ratios obtained with the *dl.* and *l.* adrenines are probably due to the same cause, depression of the excitability of the heart and arteries by the anæsthetic, or by other conditions, affecting the weaker response to *dl.* adrenine in greater degree than the more active response to *l.* adrenine. Similarly, in the other cases, the response to that one of two bases, which the more nearly approaches adrenine in structure and activity, is more resistant to depressing influences of various kinds than the response to the other. In classes (*b*) and (*c*) the response to the amino-base falls off more rapidly than that to the methylamino-base, even when the former is initially the more potent stimulus. To use a metaphor, which, however, must not be interpreted too literally, the discrimination of the excitable tissue in favour of the configuration of its natural stimulant (adrenine) appears to become more severe as its avidity diminishes.

It will be clear that under such conditions an accurate estimate of the relative activities even of corresponding members of groups (*a*), (*b*) and (*c*) is not possible. Methylamino-ethyl-catechol, the most active of those outside group (*c*), was compared with *dl.* adrenine and with the natural *l.* base, ratios of 1 : 7 and 1 : 10 being obtained by finding, as usual, doses causing rises of pressure to equal submaximal summits. Equal summits do not, however, indicate equal total effects, since the effect of the ethyl-catechol-base lasts considerably longer than that of adrenine, with doses producing equal pressure maxima. The same criticism applies to most of the comparisons between members of different groups and different series. Bearing in mind the rather arbitrary nature of the method, the approximate average activity-values by the blood-pressure method in the catechol series of bases may be tabulated as follows:—

$(OH)_2C_6H_3 . CH_2 . CH_2NH_2$ 1.
$(OH)_2C_6H_3 . CH_2 . CH_2NHCH_3$ 5.
$(OH)_2C_6H_3 . CH_2 . CH_2NHC_2H_5$ 1·5.
$(OH)_2C_6H_3 . CH_2 . CH_2NHC_3H_7$ 0·25.
$(OH)_2C_6H_3 . CO . CH_2NH_2$ 1·5.
$(OH)_2C_6H_3 . CO . CH_2NHCH_3$?
$(OH)_2C_6H_3 . CO . CH_2NHC_2H_5$ 2·25.
$(OH)_2C_6H_3 . CO . CH_2NHC_3H_7$ 0·25.
di. $(OH)_2C_6H_3 . CHOH . CH_2NH_2$ 50.
di. $(OH)_2C_6H_3 . CHOH . CH_2NHCH_3$ 35.

As mentioned above, these ratios are subject to somewhat wide variation with the sensitiveness of the animal, even when a uniform method of preparation, such as decerebration, is employed. It is not surprising, therefore, to find Schultz assigning the ratio 1 : 80 for the activities of ethylamino-aceto-catechol to adrenine, whereas our table shows a much smaller ratio.

Effects on other organs than the circulatory system.

Apart from the effects on the blood-pressure the sympathomimetic action which has been most fully studied is that on the uterus of the non-pregnant cat. For the purpose of comparing the effects of a number of the bases this is most conveniently treated as an isolated organ by Kehrer's[1] method, provision being made for repeatedly changing the Ringer's solution without disturbance of the preparation. An exact numerical comparison of activities in producing inhibition of this organ has not been possible. The differences to which we refer are, however, so marked that precise measurement is unnecessary. The factors to be taken into account in forming an estimate of the relative activities of two bases, on such a preparation, are the rapidity with which relaxation takes place, and the extent to which the normal rhythm is abolished, as well as the completeness with which the tone is ultimately inhibited.

A very wide range of sensitiveness in response is presented by different specimens of the uterus. This is to be expected from the known variety of response presented by the non-pregnant but not virgin organ to stimulation of the hypogastric nerves, a variety which, for convenience of conception, may be pictured as dependent on the presence of varying mixtures of motor and inhibitor elements in the peripheral excitable mechanism.

On testing the action of the various catechol bases on any non-pregnant cat's uterus, however, one fact at once becomes distinct, namely that the methylamino-bases have a far more pronounced inhibitor action than any of the others (Figs. 11, 12, 13). The

[1] *Arch. f. Gynäkol.* LXXXI. p. 160. 1906.

ethylamino-bases in each of the two classes, (*a*) and (*b*), in which they have been examined generally, come next in order of activity; but the order of the bases other than the methylamino-bases varies with the uterus employed. With the virgin organ, which gives a purely inhibitor response to stimulation of the hypogastric nerves, the order is, almost invariably, methylamino, ethylamino, amino, propylamino. In class (*b*) this, it will be remembered, is also the order of the pressor activities; but the superiority of the methylamino-base of class (*a*) in inhibitor action is especially noteworthy in view of its markedly inferior

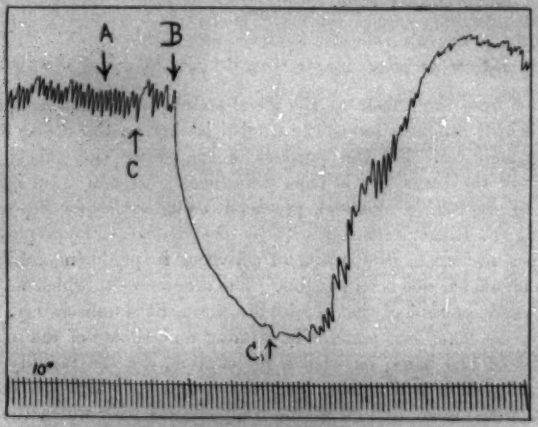


Fig. 11. Isolated uterus of non-pregnant cat.
At *A*, 0·25 c.c. $\frac{N}{100}$ amino-aceto-catechol.
B, ,, ,, methylamino-aceto-catechol.
Fresh Ringer at *C*, *C*.

pressor activity as compared with the ethylamino- and amino-bases of that class. With the non-pregnant but multiparous organ a different order is frequently observed. On such an organ the inhibitor action of the amino-bases is usually very slight, and it may even be replaced by a very weak augmentor effect on the tonus, the methylamino-bases still causing profound inhibition. In such a case as the last-mentioned it is usually found that the inhibitor action of the ethylamino-bases is also comparatively weak, and, in one such case, that of the ethylamino-ketone was distinctly inferior to that of the propylamino-ketone.

In class (*c*) only the amino- and methylamino-members were available, but here the comparatively weak inhibitor action of the powerfully pressor amino-base and the intense inhibitor action of the slightly less powerfully pressor methylamino-base were again clearly marked (Fig. 13).

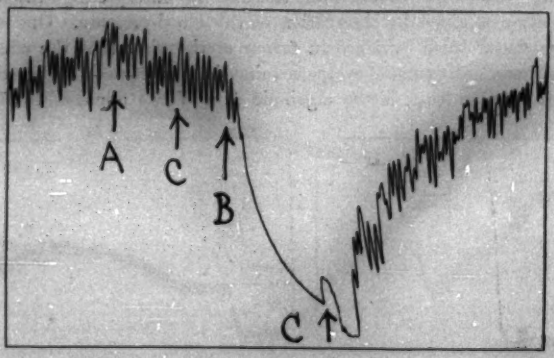


Fig. 12. Same as Fig. 11. Ethyl-catechol bases. Amino-base at *A*: methylamino-base at *B*.

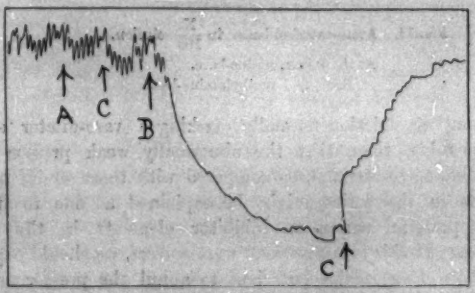


Fig. 13. Same as Figs. 11 and 12. Ethanol-catechol-bases in 0·1% solutions. One drop amino-base at *A*: 1 drop methylamino-base (*dl.* adrenine) at *B*.

In terms of the conception of the excitable mechanism as containing a mixture of motor and inhibitor elements these irregular results admit of interpretation, since we must suppose that in each case the effect produced is the algebraic sum of the two opposed effects. If we

assume that the virgin uterus gives a purely inhibitor response to all the bases, the results obtained with it represent the true order of inhibitor activity of the bases. We shall return to this point after considering the reactions of some other organs. It was evident that the exceptional position of the methylamino-bases as regards inhibitor activity must be taken into account in interpreting the order of activity obtained by observation of the blood-pressure. One of us[1] has produced some evidence in favour of the view that the effect of sympathetic nerves and of sympathomimetic substances on the arterioles, of carnivora at least, is the algebraic sum of two opposed effects—a

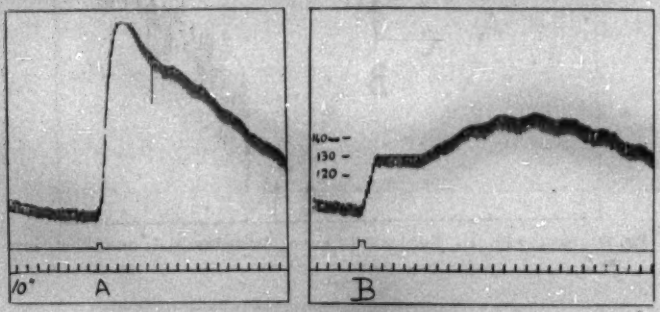


Fig. 14. Aceto-catechol bases in $\frac{N}{100}$ solution.
At *A*, 0·5 c.c. amino-base.
B, „ methylamino-base.

predominant constriction normally masking a vaso-dilator effect. It seemed possible, then, that the abnormally weak pressor effect of methylamino-aceto-catechol, as compared with those of its immediate neighbours in the series, might be explained as due to its proved relatively powerful action on inhibitor elements in the excitable mechanism. If this interpretation were correct, we should expect that, after a dose of ergotoxine sufficient to annul the pressor action, the methyl-amino-ketone would produce a larger fall of blood-pressure, due to vaso-dilatation, than either the amino- or ethylamino-ketone. As a matter of fact we found it quite easy to give such a dose of ergotoxine that, while the amino-ketone still caused a small rise of pressure, an equivalent dose of the methylamino-ketone produced a large purely

[1] Dale. This *Journal*, xxxiv. p. 163. 1906.

depressor effect (see Figs. 14 and 15). On the same lines it might be suspected that the apparently weaker pressor action of *dl.* adrenine, as compared with *dl.* amino-ethanol-catechol, was attributable to the greater relative affinity of the former for the inhibitor element in the excitable mechanism. This can again be verified with the aid of ergotoxine, moderate doses of which leave the amino-base with a remnant of pressor action, while that of the methylamino-base (adrenine) is replaced by depressor action.

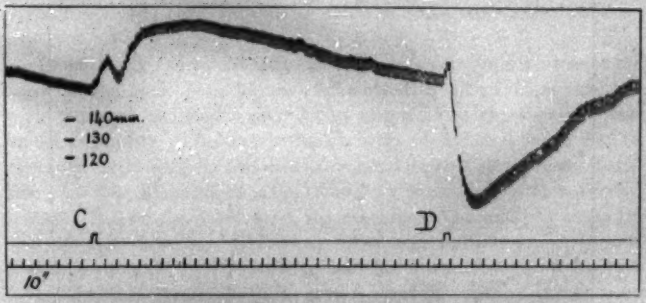


Fig. 15. Same experiment at 14, after 2 mgms. of ergotoxine phosphate.
At *C*, 0·5 c.c. amino-base.
D, „ methylamino-base.

It will be clear that, if we are right in the supposition that the receptive mechanism of the arterioles contains inhibitor as well as motor elements, an organ which gives a purely motor response to these bases should give us yet another order of activity for the ketones. There is reason to expect such a purely motor response from the retractor penis muscle of the dog[1]. The motor effect of stimulating the sympathetic nerve-supply, or of adrenine, on this muscle is gradually extinguished by ergotoxine, but never, in our experience, replaced by relaxation. For comparison of the actions of the bases we treated it as an isolated organ.

The effects on it of the amino-, methylamino-, and ethylamino-ketones, are closely similar to one another, but the methylamino- is almost identical in activity with the amino-ketone (Fig. 16), the

[1] We obtained closely similar results in one experiment on the retractor penis of the goat.

ethylamino- rather more active than either. The propylamino-ketone is far less active than the other three.

Of the bases in group (*b*), derivatives of ethyl-catechol, the methylamino-base has by far the most powerful action on the retractor penis, the ethylamino-, amino-, and propylamino-bases following in that order of activity. This order, it will be seen, is identical with that obtained with the blood-pressure, and the non-pregnant uterus.

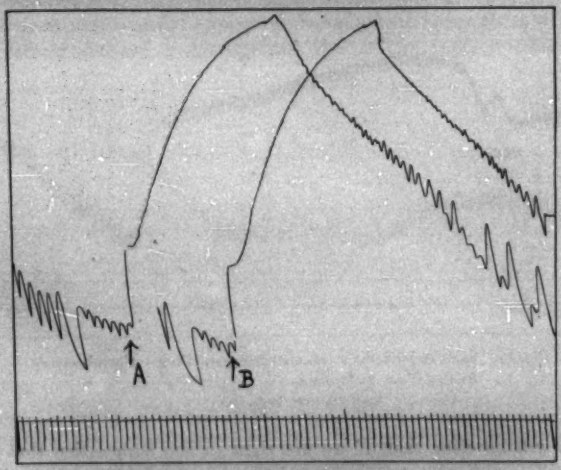


Fig. 16. Isolated retractor penis of dog in 250 c.c. Ringer's solution.
At *A*, 0·5 c.c. amino-aceto-catechol.
B, ,, methylamino-aceto-catechol.

In group (*c*) it is interesting to find that the methylamino-base (adrenine) is markedly more active on the retractor penis than the amino-base, the *dl.* form being used in both cases.

It is quite possible that yet different orders of activity might be obtained by experiments on other organs innervated by sympathetic nerves. We have confined our attention to these for this purpose since they provide instances of what we regard as purely motor, purely inhibitor, and mixed responses. It will be clear that, in the last case, the order of activity in a particular direction may be expected to show variations if the proportion of the motor to the inhibitor factor in the response varies. This is known to be the case with the non-pregnant

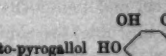
but not virgin cat's uterus. Such variations of order would be particularly probable in the case of the ketones, in which the orders of activity on organs with purely motor and purely inhibitor responses are not identical. It is, indeed, in the ketone group that the variations of order with different examples of the non-pregnant uterus are observed.

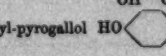
It may be said, then, that the view which regards the effect of these bases on most organs as the algebraic sum of two opposed effects is in accordance with the known facts, the apparent irregularities and variations in the order of activity being to some extent explicable on this view.

It has been mentioned that the ratios between the activities of the bases on the blood-pressure vary not only with different animals, but with the same individual at different stages of an experiment. This unequal rapidity of decline in the activity of different bases can be observed very readily in the case of isolated organs. As already stated the exact determination of ratios of activity is not easy in such circumstances: but, on the other hand, the experiment can easily be extended over several days, in which time differences initially slight become greatly accentuated. One striking instance will suffice. A virgin cat's uterus, isolated on April 15th, gave a marked inhibitor response on that day to *p.* hydroxyphenylethylamine and its alkylamine homologues, as shown in Fig. 5. It was kept in cold oxygenated Ringer's solution till April 18th, when, on warming again to 38°, it resumed an active rhythm. It had now, however, completely lost its sensitiveness to the bases with one phenolic hydroxyl, 5 c.c. of $\frac{N}{100}$ solutions producing no trace of inhibitor action. On the other hand its inhibitor response to methylamino-aceto-catechol was retained without perceptible impairment. Similar, though smaller differences in rate of activity-decline are observable within the limits of the catechol series of bases, and with the motor response of the retractor penis muscle.

E. Amines with three phenolic hydroxyls.

Only two of these have been available, both of them being amino-bases.

(1) Amino-aceto-pyrogallol 

(2) Amino-ethyl-pyrogallol 

They were tested on the blood-pressure and the isolated uterus of the non-pregnant cat. Both show sympathomimetic activity, but their pressor action is in each case somewhat weaker than that of the corresponding catechol base, their inhibitor action not greater. The introduction of the third hydroxyl group, therefore, in the first position relatively to the side-chain, while greatly lessening the resistance to oxidation, has not increased the sympathomimetic activity in any direction.

Some theoretical considerations.

We have already pointed out that most of the few provisional generalisations, based by previous observers on the examination of a small number of substances, for the most part closely related to adrenine, break down when the enquiry is extended over a wider field. A catechol nucleus is not an essential constituent of the molecule of a sympathomimetic substance, nor has catechol itself an action of the sympathomimetic type. It is true, however, that among the catechol bases are the most active in this direction of those which have hitherto been prepared. The fact, moreover, that the two pyrogallol bases examined were not more active than their catechol analogues suggests that one optimum condition for this type of activity is the presence of the catechol nucleus.

The suggestion that activity increases with diminishing resistance to oxidation needs more careful consideration. Broadly it is true, but it needs much qualification when applied to the individual members of an homologous series. If the pressor activity be accepted as the index the generalisation at once breaks down when so applied. We have shown, indeed, that the activity in producing motor effects, within the limits of such a series as that of the aceto-catechol bases, by no means runs parallel to the activity in producing inhibitor effects. A parallelism with instability or reducing action cannot, therefore, hold good for both types of activity. At first sight there seems some correspondence between instability and inhibitor potency in each group of catechol bases examined. Ewins[1] recently compared these bases with regard to reducing action, and found that the methylamino-base in each group was the most unstable, the amino-base the most stable. In both groups in which they were available the ethylamino- and propylamino-bases occupied an intermediate position as regards insta-

[1] This *Journal*, xi. p. 817. 1910.

bility. If the propylamino-bases be left out of consideration the same order holds for the inhibitor activity of the bases, as long as comparison is confined within the limits of an homologous series. But the necessity for the omission of the propylamino-bases weakens the significance of the comparison, which further breaks down completely when comparison is made between members of different series. Thus the comparatively stable methylamino-aceto-catechol has a more powerful inhibitor action than the much more easily oxidised methylamino-ethyl-catechol: on the other hand the amino-bases of the pyrogallol series, which are very readily oxidised, have no more inhibitor activity than the comparatively stable amino-bases of the catechol series. It must be concluded, therefore, that instability to oxidising agents is at most a subsidiary factor in determining sympathomimetic activity.

The generalisations which can be made from our results are, indeed, but few. All the substances in which we have traced the sympathomimetic action are bases. All those which show it characteristically and certainly are, moreover, primary and secondary amines. The quarternary ammonium bases, corresponding to the sympathomimetic amines of the phenolic and catechol series, have a marked activity of a quite different type, closely resembling that of nicotine. In a general way it can be stated, that approximation to adrenine in structure is attended by increase of sympathomimetic activity. The optimum carbon-skeleton, of a primary or secondary amine, for the production of this type of activity, appears to consist of a benzene ring with a side-chain of two carbon atoms, the amino-group and the benzene nucleus being attached each to a different carbon atom of this side-chain. The activity is increased by phenolic hydroxyls in the 3 : 4 position relative to the side-chain. When these are both present, but only then, the introduction of an alcoholic hydroxyl, attached to that carbon atom which is linked to the ring, further increases the activity. Inhibitor and motor activity vary to some extent independently when a hydrogen atom of the amino-group is replaced by various alkyl radicles.

Taking the last point first, it is clear that our results are compatible with such a proximate conception as that of Elliott[1], which, without involving any theory of the intimate nature of the action, postulates the existence of a myoneural junction, the function of which is to receive the nerve-stimulus or chemical stimulant substance, and to which must be ascribed the determination of the response in the

[1] *loc. cit.* and This *Journal*, xxiv. p. 367. 1907.

direction of augmentation or inhibition. If such a conception be regarded as adequate, our results merely emphasise the necessity for supposing that the myoneural junctions, or parts of myoneural junctions concerned with inhibition are not identical, in their affinities for chemical substances, with those which are concerned with motor effects. Evidence in the same direction had been previously furnished by the selective paralysis of motor sympathetic effects by ergotoxine.

Another suggestion, for which Elliott[1] was originally responsible, may be considered here, namely, that the parallelism between the actions of adrenine and of sympathetic nerves may be due to the fact that the latter produce their effects by liberating adrenine at their endings. This has more recently been adopted and propounded in greater detail by Dixon and Hamill[2], who suppose that the immediate effect, not only of nervous impulses, but also of drugs, is to liberate the appropriate hormone, which, in the case of sympathetic nerve-impulses, and of drugs reproducing their effects, is adrenine. The difficulty of applying this conception to the action of the amines which we have examined will be obvious. It would be fanciful to assume that the base, differing from adrenine merely in the fact that its amino-group is not methylated, acts not directly, but by liberating its homologous and not more active neighbour. If, however, we concede to amino-ethanol-catechol the power of acting directly, we cannot reasonably invoke the liberation of adrenine to explain the action of bases one stage further removed from it in structure, and, continuing the argument, we are ultimately bound to admit even the primary fatty amines to the list of substances acting directly, like the hormone adrenine. If these be admitted, it becomes difficult to conceive why the mediatory action of adrenine should be called in for any substance whatever. The distinction drawn between the action of a hormone and that of a compound foreign to the body, but producing similar effects, breaks down inevitably when a continuous series is available.

The conception of sympathetic nerve-impulses as acting by the liberation of adrenine seems to us unsatisfactory for another reason. It involves the assumption of a stricter parallelism between the two actions than actually exists. Adrenine has, in common with the other methylamino-bases of the catechol group, the property of exaggerating inhibitor as compared with motor effects. The inhibitor effects of these

[1] This *Journal*, XXXI. p. 20 (*Proc. Phys. Soc.*). 1904. [2] XXXVIII. p. 314. 1909.

methylamino-bases are relatively prominent not only as compared with those of homologous bases, in particular the amino-bases, but also in comparison with those of sympathetic nerves. All the sympathetic effects which are weakly or doubtfully reproduced by adrenine are motor effects—*e.g.* pilo-motor action, or contraction of the trigone of the cat's bladder. On the other hand certain inhibitor effects, such as inhibition of the fundus of the cat's bladder, or of the non-pregnant uterus of the same animal, are more easily and completely produced by adrenine than by nerve-stimulation. Similarly certain normally motor effects of adrenine are reversed by smaller doses of ergotoxine than are needed for the reversal of the corresponding motor effects of stimulating sympathetic nerves. In these respects the action of some of the other bases, particularly of the amino- and ethylamino-bases of the catechol group, corresponds more closely with that of sympathetic nerves than does that of adrenine. To suppose that such bases and sympathetic nerve-impulses alike owe their action to the liberation of adrenine seems to us to create additional difficulties of conception.

Apart from such proximate conceptions our results have some interest in relation to the current controversy as to whether the activity of these and other bases depends ultimately on a physical or chemical process. It is, perhaps, desirable to point out, in the first place, that there is no reason why the whole of the facts should necessarily be interpreted in terms of the one class of action or the other. The most striking feature in the action of the bases under consideration is the more or less strict localisation of their action to cells innervated by a certain morphological system, on which cells they act when present in very high dilutions. There must evidently be something in those cells, or connected with them and them only, which has a strong affinity for these amines. But the property on which this affinity depends is by no means necessarily or even probably that which confers stimulant activity on the amines. In brief, the stimulant activity may depend on one property, the distribution of the action on quite a different property. The existence of pairs of bases, such as atropine and pilocarpine, the action of which has a practically identical distribution, though one paralyses where the other stimulates, makes it, indeed, impossible to regard the localisation of the activity and the existence of the activity as due to the same property. Langley[1] suggests that "it is conceivable that combinations should be formed by poisons which have no effect observable by our present methods." If

[1] *Journ. of Physiol.* xxxiii. p. 409. 1905.

we are right, however, in suggesting that distribution in the body and activity depend on distinct properties, the wider possibility is open that physiologically inert substances may exist, which have a similar affinity for the structures on which, for example, atropine and pilocarpine act, but that this affinity and the resultant localisation escape recognition for lack of the property conferring physiological activity. In the case of the sympathomimetic bases, the fact that these possess stimulant properties may depend on some quite simple chemical attribute. This would be the sole factor in their action on a tissue for which they have no specific affinity. If the bases were tested, for example, on frog's skeletal muscle, in the comparatively concentrated solutions in which they affect it, it is not improbable that their activities would be found proportional to some such property as their strengths as bases. Again the action of one base, even on a sympathetically innervated tissue, would very probably be found to vary with conditions, such as temperature, in such a manner as to show that the act of stimulation depends on a chemical reaction. Hill's[1] recent mathematical investigation of the contraction-curve of frog's muscle in response to nicotine under varying conditions leads him to such a conclusion. But it by no means follows that the peculiar distribution of the action of nicotine or of the sympathomimetic amines depends on the existence of specific chemical receptors in the cells peculiarly sensitive to them, as supposed by Langley[2]. Stimulation may be a chemical process: but the fact that certain cells are preferentially stimulated by a certain group of substances, such as our amines, may mean that in those cells these substances easily reach the site of action; a supposition which is in accordance with the view advanced by Straub[3].

Our results provide no decisive evidence in one direction or the other. The theory of receptive side-chains is, indeed, very difficult to apply to our results with such precision as ought to be possible in the case of simple substances of known constitution. If the relation between the receptive substance and the active base is to be regarded as one of ordinary chemical union, such points of constitution as are common to all the bases should give some indication as to the chemical nature of the side-chain which affords them anchorage. But the only complex common to all the bases is the group , which exists in innumerable bases having no sympathomimetic activity. The theory

[1] This *Journal*, XXXIX. p. 861. 1910.
[2] *Pflüger's Archiv*, CXIX. p. 197. 1907.
[3] This *Journal*, XXXIX. p. 285. 1910.

further affords no means of interpreting the somewhat irregular variations of activity in the series, in many cases corresponding to variations of no known chemical characteristic.

From this point of view the alternative conception, which makes the activity primarily dependent on physical properties, in virtue of which these bases rapidly enter certain cells even from very high dilutions, has the advantage of making activity dependent on properties such as solubility, the laws connecting which with chemical structure are so complex and imperfectly known as to give scope for irregularity. The advantage is obviously one which further knowledge may destroy. One theory of this kind would make the affinity of certain cells for these bases depend on the presence in their limiting membranes of a highly specific solvent, which leads to the extraction of the bases from dilute watery solutions and brings them into relation with the responsive constituents of the cell, corresponding to the chief substance of Langley. On such a view the degree of activity of a sympathomimetic amine, that is, the dilution in which it produces an effect of a given intensity, would clearly depend on its partition coefficient between the specific solvent, on the one hand, and the body fluids, or other solution in which it is presented to the tissue, on the other. On the lines of the suggestion made above, however, it would also be dependent on the degree in which it possesses the other, probably chemical characteristic, in virtue of which it has a stimulant action. The existence of two independent variables would give further scope for apparent irregularities.

A further advantage of a mainly physical hypothesis is that it admits more readily of individual differences, and of differences in the one individual under different conditions. It is difficult, on the theory of chemical combination with receptive side-chains, to imagine why in one cat amino-aceto-catechol should be more active than its ethylamino-homologue, while in most cats the reversed relation holds: or why at one stage of an experiment *dl.* adrenine should appear less active, at a later more active than its amino-homologue. The only possibility seems to be the assumption that receptors of different types, corresponding to the different bases, exist in different proportions and differ in their stability. But since the number of possible sympathomimetic amines is indefinitely large, it is impossible to postulate a new kind of receptive side-chain for each new base in which this type of activity is demonstrated. On the other hand a relatively small change in the composition of the solvent mixture required by the other hypothesis might easily account for such variations.

Similarly the fact that, with approximation to adrenine in structure, the activity not only increases in intensity but becomes gradually more strictly limited to structures innervated by sympathetic nerves, is more easily explicable on the physical hypothesis. On the other hand there is no positive evidence of any weight in favour of solubility in the ordinary sense as the important factor. The relation of the receptive mechanism to the base may well be one of solid solution or adsorption, and therefore more analogous to that of an enzyme to its substrate, as the widely different activities of optical isomers suggests.

On the whole, then, the least unsatisfactory view in the present state of knowledge seems to us to be that which regards the existence of stimulant activity as dependent on the possession of some chemical property, the distribution and, in the main, the intensity of the activity as due to a physical property.

Neither the purely chemical nor the mainly physical view satisfactorily accounts for the production of inhibition and motor action in different tissues by the same, or in the same tissue by different bases. It seems impossible at present to summarise the known facts in this direction, without having recourse to a more complicated and morphological conception such as that of Elliott, and further regarding his myoneural junction as multiple or composite, the receptive substances or solvents, in the portions concerned with inhibition and motor action respectively, not being identical.

Summary.

The following are some of the principal conclusions from the foregoing results:

(1) An action simulating that of the true sympathetic nervous system is not peculiar to adrenine, but is possessed by a large series of amines, the simplest being primary fatty amines. We describe all such amines and their action as "sympathomimetic."

(2) Approximation to adrenine in structure is, on the whole, attended with increasing intensity of sympathomimetic activity, and with increasing specificity of the action.

(3) All the substances producing this action in characteristic manner are primary and secondary amines. The quarternary amines corresponding to the aromatic members of the series have an action closely similar to that of nicotine.

(4) The optimum carbon-skeleton for sympathomimetic activity consists of a benzene ring with a side-chain of two carbon-atoms, the terminal one bearing the amino-group. Another optimum condition is the presence of two phenolic hydroxyls in the 3:4 position relative to the side-chain: when these are present, an alcoholic hydroxyl still further intensifies the activity. A phenolic hydroxyl in the 1 position does not increase the activity.

(5) Catechol has no sympathomimetic action.

(6) Motor and inhibitor sympathomimetic activity vary to some extent independently. Of the catechol bases those with a methylamino-group, including adrenine, reproduce inhibitor sympathetic effects more powerfully than motor effects: the opposite is true of the primary amines of the same series.

(7) Instability and activity show no parallelism in the series.

THE GASES OF THE ARTERIAL AND VENOUS BLOOD OF THE CAT.

By GEORGE A. BUCKMASTER and J. A. GARDNER.

(*From the Physiological Laboratory, University of London.*)

In the course of an investigation on the composition of the blood-gases under chloroform-anæsthesia we found it desirable to have data as to the composition of arterial and venous blood of cats which had not been anæsthetised, or anæsthetised by a non-volatile anæsthetic. The majority of published analyses are those of dog's blood and how far these results could be applied to cat's blood was uncertain. The existing analyses are comparatively few, and such data as are available have been collected from experiments made more than forty years ago, before the blood pump had reached the high degree of efficiency attained in modern instruments. According to P. Hering[1] from the results of six experiments, phosphoric acid being employed to liberate the fixed carbon dioxide, the following amounts of gas were found in arterial blood:

CO_2	28·8
O	13·1
N	1·3

Method.

Arterial blood. In some of the experiments the animals were killed by a blow on the head and the sample of blood drawn from the carotid or femoral artery while the heart was still beating and the respiration, as tracings showed, was proceeding in a normal manner. The methods for collecting the blood and introducing this into the pump are fully

[1] Quoted from Pflüger, *Arch. f. d. ges. Physiol.* 1868, p. 274.

described in our paper on a new form of blood-gas pump[1]. In some experiments the first method was employed of running the blood through a capillary cannula *direct from the artery* into the froth-chamber; in others, the second method was used, and the arrangement of the apparatus employed is illustrated in Fig. 2, p. 375 of the above paper. The cannula was introduced into the vessel immediately before the blood was withdrawn.

In another series of experiments the animals were anæsthetised by subcutaneous injections of urethane, 9 grams in 9 c.c. of water for a cat of three kilograms. A solution of hirudin (10 c.c.) was injected into the femoral vein about a quarter of an hour before the withdrawal of blood. The second method was employed in these cases. In animals treated in this way the respiratory tracings showed that the respiration was slower and shallower than in animals which had been killed by a blow.

Venous blood. In this case the animals were always anæsthetised by urethane, injected with hirudin and blood taken from the jugular vein by a long cannula which reached the right auricle. The second method was adopted.

The pump used had neither taps nor joints. It is fully described in the paper already quoted and was used with all the precautions mentioned therein, the gases being finally evacuated at 40° C.

The gas analysis apparatus employed was the well-known form of Frankland, as modified by W. A. Bone, the measuring tube and the barometer being moistened with 20 p.c. sulphuric acid. The readings were taken with a cathetometer.

The results of our analyses are given in the following tables, and the protocols are appended.

TABLE I. *Analyses of arterial blood, the volume in c.c. at* 0° C. *and* 760 *mm.*

No. of Exp.	Weight of Cat (kilos)	Source of blood	Total gas	Carbon dioxide	Oxygen	Nitrogen
I.	3·2	Carotid	39·34	25·81	12·70	0·83
II.	2·2	Carotid	28·59	15·79	11·97	0·83
III.	3·0	Carotid	33·73	18·54	14·18	1·00
IV.	—	Carotid	38·41	22·11	15·13	1·17
V.	3·2	Femoral	50·07	34·52	14·50	1·05
VI.	2·7	Femoral	47·91	33·68	13·10	1·14
		Mean	39·68	25·07	13·60	1·00

[1] *Journ. of Physiol.* XL. p. 373. 1910.

TABLE II. *Analyses of venous blood, the volume in c.c. at 0° C. and 760 mm.*

No. of Exp.	Weight of Cat (kilos)	Source of blood	Total gas	Carbon dioxide	Oxygen	Nitrogen
VII.	3·7	Right Auricle	56·66	44·24	11·31	1·12
VIII.	3·0	Right Auricle	46·39	37·42	8·54	0·42
		Mean	51·53	40·83	9·93	0·77

We also include a Table from observations made with a Toepler-Barcroft pump, which had been in use for a considerable time. The nitrogen-values were between 1·5 and 4, and have been adjusted to 1·00, in order to make the figures comparable with Table I.

TABLE III. *Analyses of arterial blood, the volume in c.c. at 0° C. and 760 mm.*

No. of Exp.	Weight of Cat (kilos)	Source of blood	Total gas	Carbon dioxide	Oxygen	Nitrogen
A.	3	Carotid	32·40	17·50	13·85	1·00
B.	3·6	Carotid	34·14	17·79	15·37	1·00

The results obtained by P. Hering, whose original paper is an Inaugural Dissertation which we have been unable to find, are at times wrongly ascribed to Pflüger[1]. The mean values given for carbon dioxide are in close agreement with our own, and as is the current opinion, while the oxygen content of both arterial and venous blood remains approximately constant, large variations occur in the amount of carbon dioxide. The nitrogen-values are in our experiments uniformly lower and indeed lower than the analyses of blood gases of other animals published by other observers, but our values, though small, do actually show for the arterial blood of cats a variation of nearly 30 p.c.

Protocols.

Cats killed by a blow on the head.

I. 3·2 kilos. 10 c.c. of hirudin injected. Blood withdrawn, somewhat dark in colour. Volume of blood 55 c.c. Pressure of gases at a constant volume (72·48 c.c.) = 23·75 cm. at 12·8° C.: after absorption with potash (40 %), 8·2 cm. at 13·9° C.; after alkaline pyrogallate, 0·5 cm. at 13° C.

II. 2·2 kilos. Blood withdrawn rather dark in colour. Volume of blood 55 c.c. Pressure of gases at a constant volume (72·48) = 17·19 cm. at 11·6° C.: after absorption with potash (40 %), 7·7 cm. at 11·6° C.; after pyrogallate, 0·50 cm. at 11·6° C.

[1] *Dict. de Physiologie*, article Chat, p. 333, t. III. 1898.

III. 3·0 kilos. 10 c.c. of hirudin injected. Blood withdrawn, bright red in colour. Volume of blood 55 c.c. Hæmoglobin value (Gowers-Haldane) 70 %. Pressure of gases at a constant volume (72·48 c.c.) = 20·30 cm. at 12° C.: after absorption with potash (40 %) 9·1 cm. at 10·8° C.; after pyrogallate, 0·6 cm. at 10·8° C.

IV. 10 c.c. of hirudin injected. Weight of blood 49·3 grammes. Sp. G. 1054. Hæmoglobin (Gowers-Haldane) 92 %. Pressure of gases at a constant volume (72·48 c.c.) = 19·75 cm. at 13·2° C.: after potash, 8·40 cm. at 13·8° C.; after pyrogallate, 0·60 cm. at 12·1° C.

Cats anæsthetised with urethane.

V. 3·2 kilos. 5 grams of urethane (subcutaneous). Hirudin 10 c.c. injected into femoral vein. Sample taken from femoral artery. Volume of blood 21·5 c.c. Pressure of gases at a constant volume (48·65 c.c.) = 18·05 cm. at 20° C.: after KOH 5·60 cm. at 19·7° C.; after pyrogallate acid 0·38 cm. at 20·1° C.

VI. 2·7 kilos. 8·6 grams of urethane injected subcutaneously. Hirudin 10 c.c. injected into femoral vein. Sample of femoral artery. Volume of blood 21·44 c.c. Pressure of gases at a constant volume (48·65 c.c.) = 20·12 cm. at 19·3° C.: after absorption with potash, 5·10 cm. at 19·5° C. After absorption with pyrogallate, 0·41 cm. at 19·6° C.

VII. 3·7 kilos. 13 grams of urethane. Hirudin 10 c.c. injected into the femoral vein. Sample from the right auricle. Volume 21·5 c.c. Pressure of gases at a constant volume (48·65 c.c.) = 20·28 cm. at 17·9° C.: after potash, 4·45 cm. at 18·1° C.; after pyrogallate acid, 0·40 cm. at 18·2° C.

VIII. 3 kilos. 7 grams of urethane. Sample of blood taken from the right heart 21·5 c.c. Pressure of gases at a constant volume (48·65 c.c.) = 16·60 cm. at 17·9° C.: after absorption with potash, 3·21 cm. at 18·1° C.; after absorption with pyrogallate, 0·15 cm. at 18·5° C.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for aid in carrying out this work.

AN INVESTIGATION OF THE SINO-AURICULAR NODE OF THE MAMMALIAN HEART. By MARTIN FLACK.

(*From the Hallerianum, Berne.*)

In tracing the form and nature of the muscular connections between the primary divisions of the vertebrate heart, the writer, together with Prof. Arthur Keith[1], described a remarkable remnant of primitive fibres which persists at the sino-auricular junction in the mammalian heart. Many human hearts, fœtal normal and pathological, were examined as well as the hearts of such divergent species as the mole, porpoise, dolphin, kangaroo, wallaby, whale, mouse, shrew-mouse, rat, kitten, ram, pig, cart horse, pony, and fœtal gibbon. In all these hearts was this remnant of the sinus of the primitive heart found. It is now known by the name of the "Keith-Flack node" or better the "sino-auricular node." In structure it resembles that of the already known 'auriculo-ventricular node" (A-V node) or "node of Tawara[2]."

The sino-auricular node lies in the sulcus terminalis just below the fork formed between the junction of the upper surface of the auricular appendix with the superior vena cava. Its position can be seen from the accompanying figure modified from our original paper.

As can be seen the sino-auricular node is remarkable for its special arterial supply. In microscopic sections of it the artery is found surrounded by fibrous tissue in which are numerous peculiar muscle fibres, some nerve cells and nerve fibres. Dissection in large hearts shows that the nerve cells and fibres connect with the vagal and sympathetic nerve trunks, which form a rich plexus at this junction. The musculature of the node becomes continuous with that of the superior cava, auricle and auricular canal. In describing the node in the heart of the mole we said: "Although the mass by its connections

[1] *Journ. of Anat. and Physiol.* XLI. p. 172. 1907.

[2] S. Tawara. *Das Reizleitungssystem des Säugetierherzens.* G. Fischer, Jena, 1906.

is undoubtedly muscular, the nerves in the neighbourhood of the superior vena cava appear to come into very intimate connection with it, so much so that we feel justified in stating that a highly differentiated neuro-muscular junction occurs at this point." We suggested also that the dominating rhythm of the heart normally arises in this spot.

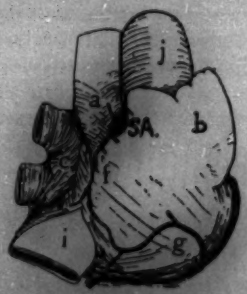


Fig. 1. Right auricle seen from the side. *a*, superior vena cava; *b*, appendix; S-A, sino-auricular node; *c*, vestibule of left auricle; *f*, union in sulcus terminalis of two branch arteries arising from right coronary artery; *g*, another anastomosing branch from right coronary artery; *i*, inferior vena cava; *j*, aorta.

The object of this paper has been to obtain evidence of the function of this node. The work has been carried out in the Hallerianum, Berne. Prof. Kronecker has kindly helped me in the experiments and I wish to express my thanks to him for the great interest he has shown in the work and for his kind assistance and advice.

The experiments have been made upon 13 rabbits, five dogs and three cats. In most of them the movements of the right auricle and right ventricle were recorded by means of hooks, the first in the tip of the auricular appendix, the second in the middle of the right ventricle. In the first few experiments however the ventricular hook was inserted into the left ventricle. By means of these hooks the heart pulled upon two Marey tambours ("récepteurs") which transmitted their movement to a second pair (inscripteurs) which recorded upon the kymograph.

At first was ascertained the effect upon the node of such treatment as the application of cold, electrical stimulation, clamping and ligaturing.

The Effect of Cold. The effect of cold upon the node was studied (i) by the application of a thin cooled silver rod, (ii) by the perfusion of ice cold water through a small silver cannula held upon the node,

(iii) by freezing the node with ethyl chloride, the surrounding parts of the heart being carefully protected by cotton wool. The effect in all cases is the same, namely an immediate slowing of both auricles and ventricles (see Fig. 2 *A*). Only when applied at the node does cold affect the rhythm of all four chambers; applied at other points of the heart its effect is nil or purely local (Fig. 2 *B*). This confirms the observation of Adam[1] that cold can only modify the heart rhythm when applied at a point between the superior and inferior venæ cavæ. Adam undoubtedly applied cold in the neighbourhood of the sino-auricular node.

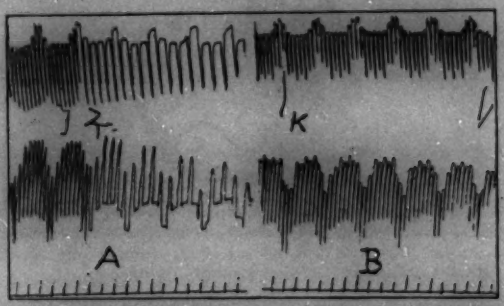


Fig. 2. *A*. At *K* cold was applied by means of ethyl chloride to the sino-auricular node. *B*. Between the strokes cold was applied to the superior cava a short distance below the node. Time in seconds in all tracings.

The Effects of Electrical Stimulation. Many experiments have been made to ascertain these effects. In all cases platinum electrodes were used, all parts except the points being isolated by rubber. In the first five experiments the Kronecker coil was used with the ordinary zinc-carbon immersion battery. In all the later ones the Guelcher's thermo-electric battery was used together with Kronecker's interrupter with the mercury "spülkontact." This interrupter has two mercury contacts one leading to the electromagnet of the vibrating rod, the other to the induction coil. The advantage of this arrangement is that the current to the electromagnet is separate from that to the coil, the current of the latter is therefore not in any way interfered with by the former and is therefore always constant[2]. The effects of electrical stimulation

[1] Pflüger's *Archiv*, cxi. p. 607. 1905.

[2] See *Zeitschr. f. Instrumentenkunde*, p. 240. 1889. Also reprint 1st *Intern. Physiol. Congress*. 1889.

may be summarised thus: (i) With weak stimulation of the node usually about 40 units Kronecker coil there occurs a marked inhibition of both auricles and ventricles (Fig. 3 *A*). (ii) With slightly higher stimulation (usually 50—60 units) is obtained a mixed effect of inhibition and acceleration of both auricles and ventricles, the acceleration usually predominating (Fig. 3 *B*). Fig. 4 also shows a mixed effect obtained in the dog. (iii) With slightly increased stimulation (60—80 units) a very marked acceleration of the whole heart rhythm is brought about. This acceleration is so marked that the recording tambours cannot follow it. The quickened beats therefore are for the most part recorded as irregular lines (Fig. 3 *C*). An example of moderate accelera-

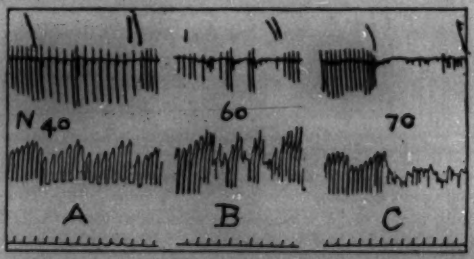


Fig. 3. *A*. Inhibition effect with 40 units. *B*. Mixed inhibition and acceleration effect with 60 units. *C*. Acceleration effect with 70 units, too quick to be recorded by tambours. Stimulation in each case between the strokes.

tion is shown in Fig. 5. (iv) With strengths above this there occurs either as before a very marked acceleration of both auricles and ventricles or an altered rhythm of auricles and ventricles during stimulation followed by a marked quickening of the heart rhythm after cessation of the stimulus. This latter may possibly be a spread effect owing to the strength of current used.

It is to be noted that in cases (i—iii) the same degree of stimulation applied to other parts of the superior cava, sulcus terminalis gives no results. When applied to the auricles the slighter degrees of stimulation give no effect (Fig. 5) or a slight quickening of the auricular beat. Stronger stimulation induces peculiar wave-like contractions of both auricles but not of the ventricles. The peculiar wave-like contractions have been called "wogen." Upon the kymograph these are recorded mostly as irregular lines, but to the naked eye they are altogether

different from the quickened *beat* induced by stimulation of the node. The latter are quick forcible little beats, the former (wogen) have but little force, the contractile power of the auricles being apparently depressed. These strengths (60—80 units) when applied to the different parts of the ventricles give no effect. A strength such as 400 units which when applied to the apex, will induce only a slight quickening, applied to Kronecker's vaso-motor centre will induce fibrillary contractions.

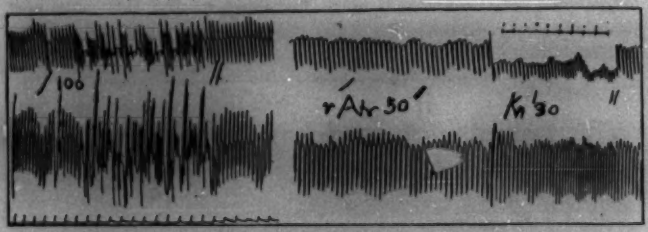


Fig. 4. Stimulation between the strokes of node in dog with $\frac{1}{10}$, 100 units. Mixed effects of acceleration and inhibition.

Fig. 5. Showing (i) no effect of stimulation of right auricle with 50 units (ii) moderate acceleration of the whole heart by stimulation of S-A node with 30 units.

These effects of electrical stimulation of the node would appear to be of great interest clinically inasmuch as stimulation of the node either through its blood supply or through its nerves may conceivably give rise to those conditions of slowed heart beat, irregularly accelerated and slowed beat, greatly quickened beat and possibly altered rhythm of auricles and ventricles.

The Effects of Clamping and of Ligaturing. The effects of clamping the node depend upon the intensity of the pressure exerted in the clamping. For this purpose either ordinary forceps or Spencer-Wells forceps were used. The effects are with *moderate* pressure (*i.e.* stimulation) (i) mostly a marked quickening of the whole heart rhythm (acceleration effect); (ii) sometimes an altered rhythm of auricles to ventricles; (iii) occasionally a slowing of the whole heart beat. The same treatment applied to other parts of the superior cava, auricles and ventricles elicits no effect. With *strong* clamping sufficient to obliterate the node little or no effect is produced upon the heart rhythm. The same may be said of ligaturing the node. This result is at first sight

astonishing. It is known that the amount of nodal tissue varies in amount in different hearts of the same species[1], and it might be that all was not included in the clamp or ligature. But the constancy of the phenomenon is against this view. Moreover during the course of these experiments my attention was drawn to the preliminary report of those of Jaeger[2]. This observer finds no altered rhythm as the result of destruction of the S-A node by burning.

It is to be remembered however that there are other remnants of the primitive sinus in the mammalian heart, more particularly in the neighbourhood of the coronary sinus, and possibly around the orifices of the pulmonary veins of the left auricles. These remnants may be capable of maintaining the rhythm even when the superior caval node is destroyed. Further evidence is required upon this point. The fact that complete destruction of the node appears to influence the heart rhythm but little, whereas its stimulation produces the important effects given above, seems to indicate that the cases of altered rhythm met with clinically and attributed to the condition of this node must be assigned rather to its stimulation than to any depression or destruction of the same. Hering[3] however states that destruction of the sino-auricular node leads to a diminution in the interval which normally elapses between the auricular and ventricular beats (A_r-V_r interval). I did not see this statement until after the completion of my experiments and have made no observations upon this point.

The following protocol will serve to illustrate some details of the method and results. Five experiments, in general similar, were made.

Exp. 1. Rabbit, Morphine, Curari.

Stimulation of sino-auricular node by induction shocks gave:

(1) With 40—50 units (Kronecker coil) a slowing of both auricles and ventricles. Upon other parts of the sulcus terminalis, upon superior cava, no effect. Upon right auricle slight quickening of beat. Upon right ventricle no effect.

(2) With 60—80 units a mixture of effects—inhibition and quickening—the accelerating effect predominating. The effect is upon both auricles and ventricles.

(3) With 80—100 units marked acceleration of both auricles and ventricles. Upon other parts of sulcus terminalis no effect. Upon the right auricle this strength caused "wave-like" contractions of both auricles. Upon the right ventricle no effect.

(4) Stronger stimuli (200—350) to node induced allorhythmia of auricles and ventricles. The same strengths applied to auricles induced fibrillary contractions; to right ventricle, at the apex slight quickening, at the site of Kronecker's "stich" (vaso-motor centre) fibrillary contractions.

[1] Keith and Flack, *loc. cit.*

[2] *Ztrlbl. f. ges. Med.* 28 May, 1910.

[3] *Münch. Med. Wochensch.* 27 Ap. 1909.

Cold applied to node by means of silver cannula slowed both auricles and ventricles (50 per minute) when applied to other parts of sulcus, to auricles and ventricles no effect.

Pinching the node with forceps gently gave a marked quickening of both auricles and ventricles (48 per minute). Pinching other parts of sulcus gave no effect.

A *ligature* round the sulcus terminalis stopped both auricles and ventricles at first—then ventricles began to beat again. Upon releasing the ligature and gently squeezing node the rhythm returned to normal.

A *ligature* of the node clamped in forceps had no effect upon rhythm.

As the effects of electrical stimulation of the node resembled those obtained by the stimulation of the vagus and of the accelerator nerves themselves, the next points investigated were to see (i) whether the node and these nerves behaved in the same manner to different rates of stimulation, (ii) whether the two sets of nerves themselves differed in their behaviour to different rates of stimulation. It was found that both the nerves and the node were more responsive to quick rates of stimulation (20 per sec., 100 per sec.) than to slow (3 per sec., 5 per sec.). In the rabbit 3 stimulations per sec. were ineffective on the vagus nerves (Fig. 6) in the dog the effect was slight, but stimulation with 1000 units was less effective than with 50 units with 20 stimulations per sec. In one experiment (Exp. 2 Table I) either something in the curari itself, or something inadvertently mixed with it, rendered the heart markedly insusceptible to both direct and nervous stimulation. Two other experiments upon rabbits afforded similar results. This curari was not then used again.

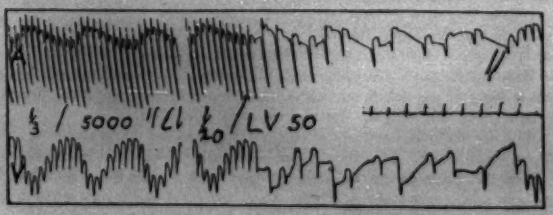


Fig. 6. Effects of stimulating vagus nerve (rabbit) (i) with 5000 units at 3 induction shocks ($\frac{1}{1}$) per sec. (ii) with 50 units at 20 induction shocks per sec. ($\frac{1}{20}$).

Experiments were also made to ascertain the relationship of the vagus and sympathetic nerves to the node. The chief points investigated were, the effect of application of atropine and muscarine to the node, the effect of stimulation of the nerves during application of cold to

the node and after tightly clamping it. The number of experiments in which drugs were used is not large, but the results obtained are interesting and in accordance with those obtained by freezing and by clamping the node.

The Effect of Atropine. To apply atropine to the node a very small piece of cotton wool was twisted around the points of a pair of fine forceps. This was then dipped into the solution (·05 %), squeezed free of all superfluous fluid and then applied to the nodal area for 1—2 minutes, the surrounding parts of the heart being at the same time carefully protected by cotton wool. It was found in all the cases tried that the effect of the right vagus nerve upon both the auricles and ventricles was abolished. As concerns the left vagus the effects varied as follows (i) the effect upon both auricles and ventricles was abolished, (ii) the effect on both auricles only was abolished, that on the ventricles at first persisting.

The effect of the drug applied in this manner is quite transitory, the nerves again exerting their normal effect after 2½—3 mins. This time corresponds with that taken when a small amount of the drug is injected into the blood.

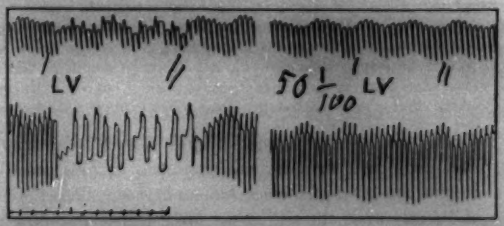


Fig. 7. Application of atropine locally to the S-A node. (i) effect of left vagus 50 units (100 induction shocks per sec.) after 1st application. (ii) effect of left vagus 50 units ($\frac{1}{100}$, *i.e.* 100 induction shocks per sec.) after 2nd application of the drug. In the first case it will be noticed that the vagus effect is abolished only on the auricles; in the second upon both auricles and ventricles the effect being equally abolished at 1000 units stimulation.

The Effect of Muscarine. This drug was applied in the same manner as atropine, a 0·5 % solution being used. The first effect is to slow markedly the rate of heart beat. At the same time the effect of the vagus nerve upon the heart is greatly increased. It will be noted that whereas without muscarine it took 50 units Kronecker coil to slow

markedly the rate of beat, after the application of the drug a greater effect was produced by 25 units (Fig. 8).

With a longer application of the drug to the node, the effect of the vagus nerve upon the heart is completely abolished, the heart continuing to beat with a slow depressed contraction. The effect of the muscarine (whether applied locally or injected into the blood stream) is abolished by the local application of atropine to the node. The primary stimulating effect of muscarine upon vagus excitability is in accordance with the observations of Asher and Glur[1] and against those of Jonescu[2]. The second effect however is in accordance with Jonescu's observations.

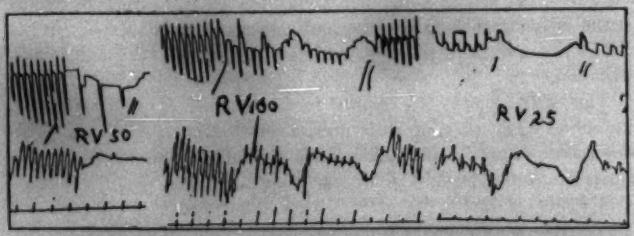


Fig. 8. Tracings showing (i) the effect of 50 units on the right vagus before the application of drugs, (ii) the effect of 100 units after the application of cold to the node, (iii) the effect of 25 units after application of muscarine. It will be seen in (ii) that the vagus still exerts an effect upon the magnitude of the contraction, but no chronotropic effect; in (iii) that the effect is more marked upon the auricles than upon the ventricles.

The Effect of the Accelerator and Vagus Nerves after application of cold. This mode of experiment was employed in three cases only, in particular to test the effect of the accelerator nerves. The ethyl chloride was sprayed directly upon the site of the node, the surrounding parts of the heart being guarded by cotton wool. In the first experiment the left accelerans only was tested, in the other cases both nerves were used. In the first two experiments it was found that freezing the node abolished the effect of the left accelerator upon the heart as also of the right accelerator in the second experiment. After these results I saw the statement of Rothberger and Winterberg[3] to the effect that in some cases (30 %) the left accelerator nerve acts upon the heart (they suggest the A-V node), after cold is applied to the great veins. Another

[1] *Ztschr. f. Biol.* LII. p. 532. 1909.
[2] *Arch. f. exp. Path. und Pharm.* LX. p. 154. 1909.
[3] *Ztrlbl. f. Physiol.* XXIV. p. 305. 1910.

dog was therefore taken and in this case it was found that whereas the action of the right accelerator was completely inhibited by the application of cold to the node (see Fig. 9) the action of the left accelerans upon the heart still continued (see Fig. 10).

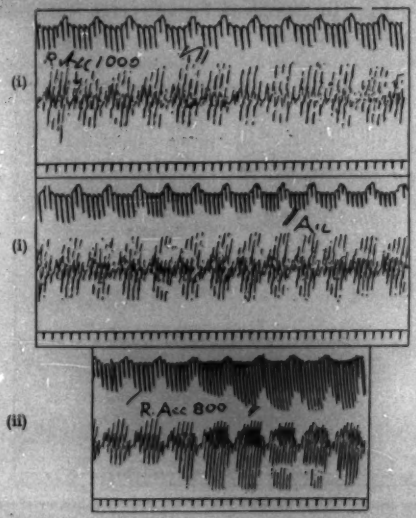


Fig. 9. Tracings to show (i) the abolition of effect of right accelerator after freezing the S-A node; the accelerator nerve was stimulated between the strokes, the freezing was discontinued at K''. Part of curve (30 secs.) has been removed. (ii) Shows action of right nerve beforehand—the action also returned after the effects of the cold had passed off.

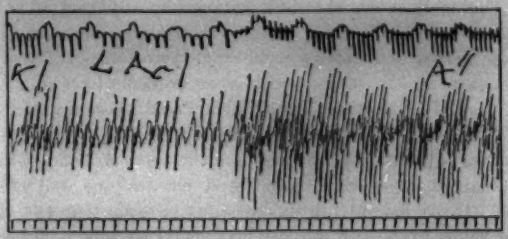


Fig. 10. To show the action of the left accelerator even after freezing of S-A node. The nerve stimulated from A'—A'', freezing started at K' and continued all the time.

As regards the effect of the vagi after the freezing of the node this was tested in two cases only. The results were after cold, (i) the right vagus no longer acted, the left continuing to act; (ii) the right vagus no longer caused chronotropic effect—but exerted its influence upon size of contraction (Fig. 8). In this heart the left nerve was almost inactive and it was not tested after cold.

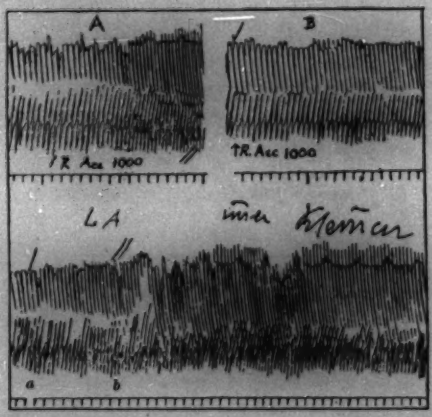


Fig. 11. The upper tracings show the abolition of the right accelerator through clamping node; A before clamping, B after clamping. The lower tracing shows in the same heart the effect of left accelerator even after clamping; stimulation of nerve from a—b.

The Effect of the Vagus and Accelerator Nerves after clamping the Node. This method was the most extensively employed as being the least open to objection. After carefully ascertaining the strengths with which the nerves undoubtedly manifested their action the area of the S-A node was tightly clamped with Spencer-Wells forceps, the clamp being left on. It is worthy of note that in one experiment an ill-fitting pair of forceps still allowed the right vagus nerve to act—on reclamping with a better pair of forceps the action of the nerve was abolished. The results obtained agree with those found for atropine and are more complete. It was found (i) in six cases out of seven that the effect of the right vagus upon both auricles and ventricles was abolished; the exception occurred in the dog (Exp. 11); it may be however that the

TABLE I. *The following table gives the results of the methods of treatment of the S-A Node. In most of the experiments also the excitability of the node and adjacent parts was tested as in Exp.* 1 *supra and with like results.*

Number of Experiment Animal		Treatment of S-A Node	Effect of Vagi on heart	Effect of accelerators on heart	Remarks
2	Cat	? over dose curare	abolished	—	Node inexcitable up to 600 units.
3	Rabbit	Clamping	R abolished A and V*	—	L vagus did not act on this heart.
4	Dog	Ethyl Chloride	R abolished A and V L still acts A and V	L abolished R not tried	L vagus did not act well to any strength of stimulation.
5	Rabbit	Clamping	Both abolished A and V	—	—
6	Rabbit	Atropine	At first on A abolished only, then on A and V (Fig. 6) both nerves	—	Muscarine to node produced slowing whole heart. Atropine to node abolished this effect.
7	Dog	(1) Atropine (2) Clamping	Both abolished A and V R abolished A and V L not tried	—	Effect of atropine passed off in three minutes.
8	Rabbit	Atropine	L abolished A and V	—	Atropine applied to node after muscarine, effect of latter abolished; also effect of injected muscarine. R vagus did not act on this heart.
9	Rabbit	Muscarine	(1) R increased A and V then (2) R abolished A and V	—	Abolished by atropine to node. L vagus did not act on this heart.
10	Dog	(1) Ethyl Chloride	R no chronotropic effect A and V (Fig. 8)	Both abolished	L vagus did not act well.
		(2) Muscarine	R increased A and V	—	—
11	Dog	(1) Ethyl Chloride	—	R abolished, L *not* (Figs. 9 and 10)	? If all node can be clamped at once in the dog.
		(2) Clamping	? both unaffected	R abolished, L *not* (Fig. 11)	
12	Rabbit	Clamping	R abolished A and V L acted on V only	—	Clamp not readjusted.
13	Rabbit	Clamping	Both abolished A and V (Fig. 12)	—	Effect of right nerve not abolished at first clamping.
14	Rabbit	Clamping	R abolished A and V L abolished on A only at first, effect returning on both A and V	—	—

* R = right. L = left. A = auricles. V = ventricles.

node was too large to clamp properly although the right accelerator effect was abolished in this case: (ii) that the effect of the left vagus is variable; in most cases where the nerve was acting at first, the effect upon both auricles and ventricles was abolished, in two cases however the effect of the nerve upon the auricles was only abolished, in one of these the effect subsequently returned.

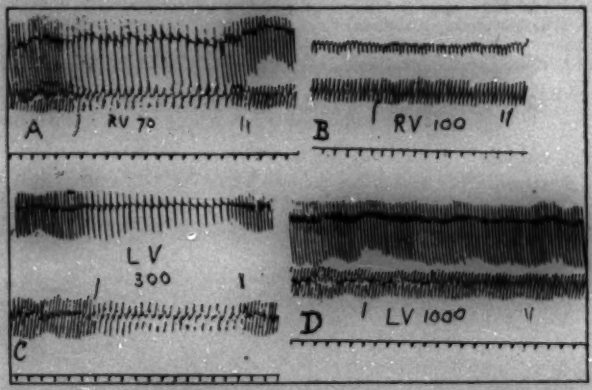


Fig. 12. *A*. effect of 70 units right vagus before clamping. *B*. effect of 100 units after clamping. There was no effect of 1000 units, the pull of the clamp has altered the tension on the tambours. *C*. effect of left vagus 300 units before clamping. *D*. effect with 1000 units after clamping. Stimulation in all cases between the strokes.

In these experiments it was found with one exception that the left vagus excited a less powerful chronotropic effect than did the right nerve, in some cases it was quite or almost inactive. It is to be noted therefore that clamping of the node always abolished the action of the nerve with the greatest chronotropic effect. The variations in the results of stimulation of the left nerve after clamping the node indicate that there is some irregularity about the manner of distribution of this nerve. On the other hand in some cases where its effect was apparently not affected readjusting the clamp has shown that such was not the case. From these few experiments it would seem that when the left vagus goes to the node, it connects with the upper region, the right nerve on the other hand connecting with the lower portion of the node.

It is unnecessary to go at great length into the work done upon the sinus venosus of the cold-blooded animals and upon the great veins

of the mammalian heart. An account of such with its literature is given by Langendorff[1] and by v. Cyon[2]. This work is connected mainly with the names of Gaskell, McWilliam, Engelmann, Langendorff and H. E. Hering. Suffice it to say that research has shown that in this region the normal heart impulse arises and that here is situated the mechanism through which the extracordial nerves exert an important effect upon the heart rhythm.

Despite the fact that obliteration of the S-A node does not apparently markedly influence the heart rhythm, the fact that here alone the application of cold and of electrical and mechanical stimuli modify the rhythm of all the heart chambers is proof sufficient that in this node the normal dominating rhythm of the mammalian heart arises. Such being the case we should expect that here too the vagus and sympathetic nerves would form a close connection. These experiments show that it is through the sino-auricular node that these nerves exert their main effect upon the heart rhythm.

Summary of Results.

(1) At the sino-auricular node alone can the normal dominating rhythm of the heart be affected (i) by the application of cold (ii) by electrical stimulation (iii) by mechanical stimulation such as pinching.

(2) At this spot the vagus and sympathetic nerves, particularly the right nerves, exert their main effect upon the heart rhythm; application of atropine and cold in most cases abolishes this effect, as does tight clamping of the node.

(3) Muscarine applied to the node slows the whole heart rhythm. It also first increases the effect of vagus stimulation and then abolishes it. Application of atropine to the node counteracts the effect of muscarine applied directly to node or injected into the blood.

[1] *Ergebn. d. Physiol.* Abt. II. 1902.

[2] *Die Nerven des Herzens*, Julius Springer, Berlin. 1907.

THE FATE OF PARAHYDROXYPHENYLETHYLAMINE IN THE ORGANISM.

By A. J. EWINS and P. P. LAIDLAW.

(*From the Wellcome Physiological Research Laboratories.*)

Parahydroxyphenylethylamine was shown by Dale and Dixon[1] to be a physiologically active substance, producing effects of the same kind as those produced by adrenine, although its activity was relatively small. This fact increased the interest of the older observations concerning its wide distribution. The base has been isolated from the products of prolonged pancreas autolysis[2], liver autolysis[3], and peptic digestion of albumin[4]. It has been isolated from cheese[5], from putrid meat[6], and putrid placentæ[7], and recently from liquid extract of ergot[8]. It is formed from tyrosine by bacterial action and it has been suggested that it may be formed by this agency in the alimentary canal. This formation has quite recently been regarded as playing a part in certain pathological states in which a high blood-pressure is the most prominent symptom. Bain[9] has adduced some evidence in favour of its excretion in the urine in such cases. The fate of the base after absorption is therefore of some interest.

With a view to determining the manner in which this substance is metabolised we have carried out experiments of two kinds.

I. Experiments on intact animals.

These consisted in the administration of a suitable dose of the base by the mouth and examining the urine collected in the following 36 hours.

II. Perfusion experiments on isolated organs.

These were performed with a view to determining the part which various organs played in the metabolism of this substance.

I. Feeding experiments.

These were performed upon dogs. On account of the physiological action of the base we were limited to the administration of comparatively small amounts. We found that 0·5 gm. of the hydrochloride was well borne by an 8 kilo dog. When this amount was given by mouth, in a gelatine capsule, no symptoms were noticed for the first half hour. During the next 10 minutes a series of symptoms developed which may be briefly described as symptoms of sympathetic stimulation. (See also Dale and Dixon, *loc. cit.*) The dog became quiet and stood in one position unless disturbed, thick saliva appeared at the angles of the mouth, and some nausea was noticeable, the hair along the back became erect so that the skin was visible between the hairs, the pupils became maximal, and the heart-beat dropped to 48 per min. and was not quite regular, being obviously under extreme vagus inhibition. Some thick mucoid saliva was vomited. These symptoms persisted for about one hour and then gradually subsided. The urine was collected for 36 hours after the administration of the amine. It was found to give a very much stronger Millon's reaction than normal dog's urine. We were able to show that this Millon reaction was due to an ether-soluble acid. This provided us with a method for isolation.

The urine collected (165 c.c.) was rendered faintly acid with acetic acid and boiled for a few minutes. The solution was then cooled, 10 c.c. of 10 % HCl added, and filtered. The filtrate was shaken out several times with ether until the ether extracts gave only a faint Millon reaction. The combined ethereal extracts were washed with a small quantity of water, the ether evaporated off and the residue taken to dryness by evaporation with alcohol. The dry product was then extracted with benzene until the extract no longer gave a reaction with Millon's reagent. The benzene solution was taken to dryness, the residue extracted with water, the aqueous solution taken to dryness and the product recrystallised from benzene. In this way we obtained 70 mgms. of an acid which melted at 147—8°. The acid was readily soluble in water, gave a strong, deep red colour-reaction with Millon's reagent, and, when it was mixed with an equal weight of synthetic parahydroxyphenylacetic acid (obtained by the hydrolysis of para-hydroxybenzyl cyanide), the melting point remained unchanged.

The urine, after extraction with ether as above, still gave a very marked reaction with Millon's reagent. Concentrated hydrochloric acid

was added so that the resulting concentration of acid was 5 %, and the urine boiled under a reflux condenser for one hour.

The solution was then again repeatedly extracted with ether. On evaporation an oily residue was obtained which gave a strong Millon reaction but from which nothing crystalline could be obtained. This residue was boiled with concentrated hydrochloric acid for about one hour, cooled, filtered, made alkaline with sodium carbonate and extracted several times with ether. The aqueous solution was then made acid with hydrochloric acid and extracted with ether until the extracts no longer gave a Millon reaction. The ether solution was then worked up for parahydroxyphenylacetic acid, as in the first part of the experiment, when 40 mgms. of acid melting at 146—7° were obtained. The total yield of parahydroxyphenylacetic acid obtained was thus 110 mgms. or 25 % of the theoretical amount possible.

Repetition of this experiment gave similar results, but in no case could we account for more than 25 % of the administered amine. This is doubtless due in part to the great difficulty in isolating a comparatively small amount of the acid from such a complex mixture as urine. It was very probable from the evidence of previous workers that, if parahydroxyphenylethylamine were quantitatively transformed into parahydroxyphenylacetic acid, we would have been able to obtain a larger amount from the urine than we actually did. Thus Schotten[30], taking 7·5 grm. of the acid himself, was able to recover 77·7 % from the urine of the next 24 hours. E. and H. Salkowski[31] administering 2 grms. to dogs recovered 40—50 %. With a view to determining what percentage could be recovered when small doses were given to dogs, we administered 0·5 grm. of the acid (as sodium salt) by mouth, and found 50 % in the urine of the next 36 hours. The amount of oxyphenylacetic acid turned out in the urine in our feeding experiments with parahydroxyphenylethylamine probably, therefore, represents about one half that actually formed during metabolism. In any case it would appear that a considerable part of the administered amine is still unaccounted for. We therefore endeavoured to trace its fate through the organism.

II. Perfusion experiments.

These experiments were performed upon isolated organs obtained immediately after death from rabbits and cats. In our earlier experiments we employed a perfusion apparatus modelled on that

used by Locke and Rosenheim[13], when studying the consumption of dextrose by the isolated heart. In our later experiments we employed a more complicated apparatus very similar to that described by Brodie[13]. The form of apparatus used made no difference to the results. Special attention was paid to three points: (1) To start the perfusion as soon as practicable after the death of the animal. (2) Thorough oxygenation of the perfusion fluid. (3) The maintenance of a constant temperature of 37°.

A. *Perfusion of liver.* In the case of liver perfusions the procedure was as follows. The animal was killed, the abdomen opened, the bile duct and hepatic artery ligatured, a cannula inserted into the portal vein, and a little saline washed in to clear the neck of the cannula from blood and thus avoid clotting. (A small tributary of the portal vein is usually present in the rabbit near the point of entry into the liver, which it is advisable to tie.) The inferior vena cava was then tied just below the entry of the hepatic veins. The inferior vena cava was cut above the diaphragm, the stomach and intestines cut away, and the liver excised along with the diaphragm. The vessels were then washed out with 100 to 150 c.c. of 0·9 % saline solution. A cannula was next inserted into the superior vena cava. The liver was transferred in an evaporating basin to the perfusion apparatus, the connections made, and perfusion commenced. The perfusion fluid used was oxygenated Ringer solution (Locke formula). It was found that a perfusion pressure of 9—11 cms. of this solution gave a steady stream from the inferior vena cava. The outflow measured from 90—100 c.c.'s. per minute. If there were no venous obstruction there was practically no leak from the liver vessels: if a temporary obstruction occurred the liver swelled up and a leak commenced. Any slight leak of this nature was soon rectified and the transuded fluid added to the venous outflow. Some trouble was experienced with frothing and it was found advisable to use as small an amount of saline to wash out the vessels as was consistent with the removal of the contained blood. Prolonged washing out of the liver vessels increases the frothing.

200 mgrs. of parahydroxyphenylethylamine hydrochloride were first circulated through and through the liver in this way. At the end of one hour and two hours samples were taken and tested physiologically. Whereas the original fluid circulated produced a considerable rise of blood-pressure, the sample taken at the end of the first hour's perfusion was almost inactive and that taken at the end of the second hour was quite inactive. The fluid throughout the perfusion gave a good Millon

reaction and towards the end of the perfusion became faintly acid. From the perfusion fluid we were able to isolate parahydroxyphenylacetic acid.

With the aim of determining the quantitative relationship of the change, 500 mgrs. of the hydrochloride of the amine were perfused for three hours, and small quantities of alkali added from time to time as the alkalinity of the perfusion fluid fell off. At the end of this time the original perfusion fluid was withdrawn and fresh Ringer substituted. This was allowed to circulate for some time. The two lots of perfusion fluid were worked up together.

No parahydroxyphenylethylamine could be detected in the fluid but 310 mgrs. of crystalline parahydroxyphenylacetic acid were isolated. In other words 70 % of the maximum possible yield of the acid was obtained from the perfusion fluid alone.

From a number of other experiments it seems that a fair sized rabbit's liver can transform about 120 mgrs. of parahydroxyphenylethylamine into the acid every hour, yielding about 100 mgrs. of oxyphenylacetic acid. Thus 750 mgrs. of the hydrochloride of the amine perfused for one hour yielded 90 mgrs. of acid, while the same amount perfused under similar conditions for two hours yielded 190 mgrs.

The isolation and identification of the parahydroxyphenylacetic acid were carried out along the same lines as those adopted for urine. The process is, however, much easier with the comparatively simple Ringer solution as compared with the highly complex and pigmented urine. The combined perfusion fluids were rendered faintly acid with dilute acetic acid and boiled. The coagulated protein was removed by filtration and the filtrate concentrated by evaporation in vacuo on a water bath to about 40 c.c. The acid solution was then extracted with ether until the ethereal extract gave only a feeble reaction with Millon's reagent. The ethereal extracts were combined, washed with a very small quantity of water and the solvent evaporated off, when the acid was obtained slightly pigmented, but well crystalline and melting at 143—5°. When it was mixed with synthetic parahydroxyphenylacetic acid, m.p. 148°, the mixture melted at 146—7°.

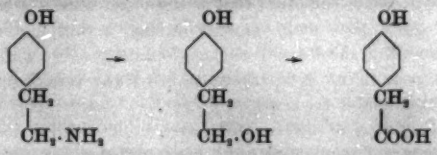
It was thought possible that this change might be brought about by liver extracts but experiments in this direction all gave negative results. 200 mgrs. of parahydroxyphenylethylamine were incubated with finely divided cats' liver for six hours without antiseptics. No parahydroxyphenylacetic acid could be detected and 80 % of the amine was recovered from the emulsion unchanged.

200 mgrs. of parahydroxyphenylethylamine were incubated with pounded rabbits' liver in the presence of chloroform for six days and no oxyphenylacetic acid could be detected. Another experiment was performed in a similar manner but the incubation period was prolonged to three weeks and the same result was obtained.

The perfusion experiments show that parahydroxyphenylethylamine is readily transformed by the liver into parahydroxyphenylacetic acid. This change may be quantitative if the perfusion be continued for a sufficient length of time. It is thus clear that the portion of the absorbed amine which is dealt with by the liver is transformed into the corresponding acid. Since any formation of parahydroxyphenylethylamine in the alimentary canal must necessarily be slow it appears improbable that any considerable quantity can pass into the general circulation unchanged. The change in all probability involves two steps

(1) Replacement of the NH_2 group by OH.

(2) The oxidation of the resultant alcohol to the acid.



We have, however, no experimental evidence in support of this intermediate step.

In our feeding experiments the symptoms of sympathetic stimulation proved that when a fairly large dose is given by mouth a portion of the amine does reach the general circulation. We have therefore endeavoured to determine the fate of this portion. For this purpose we have performed further perfusion experiments on organs innervated by the sympathetic system since the physiological action is a clear indication that some reaction occurs at these points.

B. *Perfusion of isolated uterus.* A rabbit's uterus was perfused after Kurdinowsky's method [16]. In such a small organ the rate of circulation of the perfusion fluid is necessarily slow and as parahydroxyphenylethylamine is a potent vaso-constrictor, considerable difficulty was experienced in conducting a successful experiment. The perfusion fluid (400 c.c. Ringer solution) which contained 150 mgrs. of the amine circulated very slowly through the viscus for $2\frac{3}{4}$ hours. At the end of this

time oedema appeared and the perfusion was discontinued. We were able to isolate 8 mgrs. of crystalline oxyphenylacetic acid from the perfusion fluid. The small yield is in all probability largely due to the slow rate of perfusion. It seems clear, however, that plain muscle either of the uterus or the blood vessels supplying that organ can transform parahydroxyphenylethylamine into parahydroxyphenylacetic acid.

An interesting contrast is met with in the case of perfusion of the lung. 300 mgrs. of parahydroxyphenylethylamine were perfused through a rabbit's lungs for 1¾ hours. In this experiment there was a free flow through the organ, which increases, up to a certain point, the probability of a good yield of metabolic products; and yet no trace of oxyphenylacetic acid could be detected in the perfusion fluid.

It appears to us to be probable that the mucous membrane of the uterus on the one hand, and the cells of the lung alveoli on the other, can have but little influence on the result of an experiment of this nature, and that the results are the expression of the activity of plain muscle; in the one case that of the uterus itself or the blood vessels supplying it, and in the other that of the blood vessels of the lung. The results suggest that plain muscle having a rich motor sympathetic supply can effect the transformation of amine to acid; while plain muscle having a poor or no sympathetic supply (lung vessels see Brodie and Dixon[28]) is unable to do so.

C. *Perfusion of the isolated heart.* Our third series of perfusion experiments was conducted upon isolated hearts of rabbits, cats, and one dog. The results were uniformly in the one direction, whether a large dose of parahydroxyphenylethylamine was given at the commencement of the perfusion and allowed to circulate for one or two hours, or whether a series of small doses was given at ten minute intervals. In the former case the large dose at the beginning of the experiment throws a great strain upon the heart, and the beat, after a period of greatly increased frequency and force, becomes irregular. In the latter the heart continues regular for a much greater length of time and is nearer the normal. The result is, however, the same. We found that the greater part of the added base was destroyed but that there was no corresponding amount of oxyphenylacetic acid formed. In fact, ether extraction of the acid perfusion fluids gave practically no residue and no Millon reacting substance. The hearts were also examined for amine and acid but the results were also negative. Further we were unable to detect the presence of any substance which could be considered to be a product of the katabolism of the amine, and we believe that the base is

completely destroyed or broken down into quite simple compounds. This necessitates the break up of the benzene ring and the method by which this is effected in the body is unknown.

Cardiac muscle, therefore, although capable of dealing with parahydroxyphenylethylamine does not appear to metabolise it along the same path as the liver or plain muscle. It was possible to show that parahydroxyphenylacetic acid was not an intermediate product in the degradation of the parahydroxyphenylethylamine molecule. A number of experiments were performed in which *p*-hydroxyphenylacetic acid (as Na salt) was perfused for varying lengths of time through the heart. It was always possible to recover the greater part of the acid unchanged. Some irregularity of heart beat was always noticeable in these experiments and the sodium salt of the acid appeared to exert a toxic influence. In one experiment 150 mgrs. of parahydroxyphenylacetic acid were administered in a series of small doses during two hours' perfusion of a dog's heart. Twenty-five minutes after the last dose had been given the perfusion fluid was examined for the acid and 100 mgrs. of pure *p*-oxyphenylacetic acid were recovered unchanged. In another experiment 70 mgrs. of acid were recovered from the perfusion fluid, to which 100 mgrs. had been added, and the whole perfused through and through a rabbit's heart for one and a half hours.

This distinction in the metabolic processes of plain and cardiac muscle is by itself of considerable interest, and we are of the opinion that this different method of metabolism will help to account for the comparatively small amount of oxyphenylacetic acid excreted in the urine in our feeding experiments with parahydroxyphenylethylamine. It is doubtful, however, if it will account for the whole of the difference between the amount of amine given by the mouth and the amount of acid excreted in the urine.

Having shown that parahydroxyphenylethylamine was readily transformed into oxyphenylacetic acid in the body it became of interest to determine whether methylation of the amino group would influence the direction of the metabolic process involved or the ease with which it is brought about.

We have tested this point with two different substances:

(1) The mono-methyl derivative.
(2) The di-methyl derivative.

(1) Parahydroxyphenylethylmethylamine.

For this base we are indebted to Mr G. S. Walpole[38]. As we had only a small amount at our disposal our experiments were limited

to liver perfusions. We found that the base was transformed into parahydroxyphenylacetic acid by the rabbit's liver but that the rate of destruction of the base was considerably slower than that of the primary amine. Thus 200 mgrs. perfused for two hours yielded only 65 mgrs. of acid. We have already shown that the yield of acid from parahydroxyphenylethylamine is about 100 mgrs. per hour.

(2) Parahydroxyphenylethyldimethylamine or hordenine.

With this substance we have performed two sets of experiments

(*a*) Feeding experiments.
(*b*) Perfusion experiments on rabbits' livers.

In both sets of experiments the results are very similar. 1·0 gm. hordenine sulphate was given by mouth in a gelatine capsule to a dog. No symptoms were noticed beyond nausea and one slight attack of vomiting half an hour after the administration. The urine of the next 36 hours gave a vivid Millon reaction. This was found to be due to (1) a small amount of ether soluble acid, which was too small to isolate and identify but which was in all probability oxyphenylacetic acid; and (2) unchanged base. We were able to isolate with some difficulty 50 mgrs. of base in crystalline form. This merely accounts for about 7 % of the hordenine administered; the remainder apparently disappeared.

(*b*) Perfusion of the liver gives very similar results. A trace of ether soluble acid, probably oxyphenylacetic acid, a somewhat small proportion of unchanged base, and considerable destruction.

Exp. 500 mgrs. of hordenine sulphate were perfused through a cat's liver for 2½ hours. The perfusion fluid was acidulated, boiled, filtered, and evaporated to dryness. The residue was extracted with water until the extracts no longer gave a Millon reaction, the aqueous solution acidified with HCl, and completely extracted with ether. The ethereal solution on evaporation yielded a very small quantity of residue from which was isolated a milligram or so of a substance giving an intense Millon reaction. This substance was most probably oxyphenylacetic acid.

The aqueous solution after extraction with ether was made alkaline with sodium carbonate and thoroughly extracted with ether. The ethereal solution was taken to dryness and extracted with benzene and this on evaporation yielded 35 mgrs. of crystalline hordenine.

From the foregoing experiments it seems clear that whereas the amino base *p*-hydroxyphenylethylamine is readily converted by the organism and more especially by the liver, into oxyphenylacetic acid, the corresponding methylamino base is much less readily metabolised in this direction, and with the dimethylamino base the difficulty is still further increased and little or no change takes place along this path.

On the other hand the introduction of methyl groups in this manner appears to render the base less resistant to complete destruction since in both feeding and perfusion experiments the amount of base which cannot be accounted for is progressively greater as we pass from the primary to the tertiary base.

Summary.

(1) Parahydroxyphenylethylamine is converted in part in the body into parahydroxyphenylacetic acid.

(2) The surviving liver can effect this change.

(3) Plain muscle of the uterus can effect this transformation. Plain muscle of the lung vessels cannot effect this transformation.

(4) The isolated heart causes complete destruction of the amine.

(5) Parahydroxyphenylethylmethylamine is less readily converted into oxyphenylacetic acid than the primary amine; and hordenine, the tertiary base, still less readily than the secondary base. The proportion of base unaccounted for is on the other hand greatest with the tertiary and least with the primary base.

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ON THE PRODUCTION OF GLYCOSURIA IN RELATION TO THE ACTIVITY OF THE PANCREAS.

By IVOR Ll. TUCKETT, M.A., M.D. (*Late Senior Demonstrator of Physiology in Cambridge University.*)

THE experiments submitted in this paper deal with:

(1) The behaviour of sugar injected into the circulation during different phases of pancreatic activity.

(2) The production of glycosuria during morphine and ether-narcosis in dogs.

(3) The production of glycosuria under various experimental conditions: in dogs in which a fistula of the thoracic duct was established: in cats, in which a cannula was tied into the splenic vein (through which injections were given).

Introduction. Before describing my own work I wish first to make clear in a short introduction what I regard as already proved in connection with glycosuria.

One of the chief difficulties which a study of glycosuria entails is the exceedingly voluminous literature on the subject recording a large number of observations which have been subsequently contradicted by other workers: so that it is not easy to be certain what observations already recorded are facts: and the field of research is also too big for any one worker to verify for himself all the work hitherto recorded.

But I am convinced myself that the following observations concerning glycosuria associated with hyperglycæmia may be regarded as facts[1]:

(1) That puncture of the floor of the fourth ventricle of the brain, as first observed by Claude Bernard, causes glycosuria, if the liver contains glycogen.

[1] The glycosuria produced by phloridzin is also of course a well-established fact: but as it is not associated with hyperglycæmia, I shall not refer to it in this paper.

(2) That CO-poisoning under certain conditions causes glycosuria, as shown by Straub.

(3) That narcosis caused by anæsthetics under certain conditions is associated with glycosuria.

(4) That the injection of adrenalin under certain conditions produces a pronounced glycosuria.

(5) That excision of the pancreas if properly performed is invariably followed by hyperglycæmia. (Kausch[1] showed that in ducks the sugar does not readily come out through the kidneys.)

(6) That the glycosuria of pancreatic diabetes, as Minkowski and v. Mering showed, is the result of the absence of an internal secretion of the pancreas, which, although it has never been isolated, may be regarded as a real body.

(7) That the sugar in pancreatic diabetes comes from proteid metabolism—and also from the glycogen of the liver so long as the liver contains glycogen—and passes out of the body into the urine, because neither the liver can store it nor the muscles consume it.

(8) That, as Cohnheim has shown in cats, an extract of pancreas added to an extract of muscle, with a solution of glucose added, leads to a disappearance of sugar—considerable enough to account for the metabolism of sugar in the body—under conditions where an extract of muscle alone or an extract of pancreas alone causes no appreciable loss of sugar.

These last four facts prove the existence of an internal secretion of the pancreas in the body. Therefore the probability that the internal secretion of the pancreas is a factor which has to be reckoned with in all cases of glycosuria is very great.

Now Cohnheim[2] has shown that the properties of the internal secretion of the pancreas are much more like other internal secretions (*e.g.* adrenalin and secretin), and Ehrlich's amboceptors than like enzymes or Ehrlich's complemental bodies. It is perhaps even more comparable to the body found in the liver by Magnus[3], who showed that the ester-splitting ferment of the liver can be divided into two parts, of which one is capable of withstanding moderate heat and is soluble in water and alcohol, but is insoluble in ether[1].

Therefore in cases where the internal secretion of the pancreas is a factor in the prevention of glycosuria, our mental picture of how it works may be somewhat as follows: sugar in the blood and lymph

[1] The part soluble in water and alcohol is probably a substance identical with or akin to bile salts.

owing to the presence of the internal secretion of the pancreas can be taken up by the liver and muscles and synthesised into some more complicated form of carbohydrate, being built up into the carbohydrate-proteid nucleus of the protoplasm or only loosely attached to it by a side chain. In either case the sugar disappears and is stored either in the form of glycogen as in the liver or in the form of some complex body as in the muscles, ready to break down with the production of energy. But in either case the energy of the glucose cannot be utilised by the body unless it first be synthesised into some form of carbohydrate with a larger molecule: and the internal secretion of the pancreas serves the purpose apparently of linking the glucose molecule on to the living protoplasm where it is brought into contact with the enzymes under the influence of which it is synthesised.

Now when sugar is taken by the mouth, enormous quantities of sugar if slowly absorbed can be dealt with, as there is apparently a great deal of the pancreas's internal secretion in the body: but if through excision of the pancreas or its functional derangement the coefficient of consumption of sugar in the body—principally dependent on the liver and muscles—is lowered, even a small quantity of sugar taken by the mouth appears in the urine.

Further in the normal body there is a limit to the amount of sugar which can be consumed in a given time, due perhaps in some degree to there being a limit to the quantity of the internal secretion of the pancreas in the body: although, if the percentage of sugar in the blood be much raised, it is obvious that some of it will reach the kidneys before it has had time to diffuse away and come into contact with the sugar-consuming tissues of the body.

Again, though the internal secretion of the pancreas must be present in the blood, it will be found chiefly in the lymph and tissues of the body where the metabolism of sugar takes place. Therefore the rapid introduction of sugar into the blood—either from the alimentary canal or by injection or otherwise—will lead to glycosuria because all the sugar will not have time to diffuse away and come into contact with the internal secretion of the pancreas before it reaches the kidneys.

In the first part of this paper I consider how far the facts of injection of sugar into the circulation bear out the truth of this view and if there is any evidence that normally there is much variation in the amount of the internal secretion of the pancreas in the body.

In the second part I discuss the mechanism of morphine-glycosuria in dogs and its relation to experimental glycosuria in cats.

Part I.

The following résumé of the literature connected with the introduction and injection of sugar in the body is briefly taken from a paper by Blumenthal[6].

Hofmeister was the observer who first found that the "assimilation limit" of sugar—taken by the mouth—is approximately always the same for each individual in health and each kind of sugar; and he considered that he could define an assimilation limit beyond which any sugar given appeared in the urine.

But Linossier and Roque dwelt on the fact that normal urine contains traces of sugar; and so they denied that there was such a thing as Hofmeister's "assimilation limit." They on the contrary dwelt on the importance of the "coefficient of consumption" of sugar, which shows how much sugar is used in the body if an excess of sugar is given. They found the percentage of sugar consumed decreases with the giving of increasing quantities of sugar. This result is undoubtedly partly due to sugar being so diffusible and rapidly absorbed from the intestine that much of the sugar is excreted by the kidneys before it has had time to come into contact with the tissues. Thus Donath and Schlesinger found that when dogs are fed with enormous quantities of sugar, leading to glycosuria, one very seldom finds hyperglycæmia because the kidneys excrete the excess of sugar so rapidly.

Blumenthal studied the behaviour of injected sugar and used rabbits. His method was to try how much glucose he could inject into the ear vein of a rabbit without sugar appearing in the urine which could be detected with certainty. He found that:

(1) If the injection did not last longer than ten minutes or less than one minute, the quantity of sugar which any particular individual animal could retain without any glycosuria occurring was very exact. It did not vary more than ·1 gram from time to time. (For adult rabbits of fair size the amount which could be injected without glycosuria occurring varied from 1·8 gm. to 2·8 gm. per animal according to the individual.)

(2) If the dose, which in five minutes or longer caused no glycosuria, was repeated in a subsequent five minutes, glycosuria resulted at once. (Thus in an adult rabbit in which 2·5 gm. in five minutes caused no glycosuria, 3 gm. in ten minutes caused much more marked glycosuria than 2·7 gm. in five minutes.)

From these results Blumenthal insists on the importance of what he calls the "saturation limit" of the blood, because the moment this is passed very little sugar subsequently given is retained. Thus in one experiment in which 2·6 gm. in five minutes caused no glycosuria, ·5 gm. of sugar per minute were retained and used up in the first five minutes, and later not more than ·006 gm. per minute. He also found that:

(3) If the saturation limit was once reached by giving so much sugar per minute, it only required ·02 to ·0125 of that quantity of sugar per minute which was given previously, to produce glycosuria.

(4) If sugar solutions were given very slowly during one to two hours the limit of consumption of sugar was somewhere between ·05 gm. and ·06 gm. of glucose per minute.

Blumenthal considers that the saturation limit is an indication of how much sugar can be given so that the sugar diffuses throughout all the water of the body without the percentage in the blood ever being raised enough to lead to glycosuria; whereas the "consumption limit" is reached when the liver and muscles can no longer store up glycogen or use the sugar as fast as it comes along.

Now if the consumption of sugar by the liver and muscles does not take place without the co-operation of the internal secretion of the pancreas, it is clear that the coefficient of consumption of sugar will depend partly on the activity of the pancreas, as is proved in experimental pancreatic diabetes, where the coefficient of consumption of sugar is greatly lowered. Therefore I hoped to find out if the coefficient of consumption was altered by different states of pancreatic activity, produced either by alterations of diet or by a state of fasting.

Since the pancreas is the most important gland in connection with the digestion of carbohydrates, it might be that an exclusive carbohydrate diet would have some influence on the production of its internal secretion. Thus Wienland asserted that a milk diet led after some time to the presence of a quantity of lactose in the pancreas of dogs, though this probably is not the case, since Plimmer[5] failed to confirm. Also in this connection I thought the statement of Seelig[6] to be very suggestive that ether-narcosis invariably leads to glycosuria after a meat diet but not after a carbohydrate diet: and Straub[7] found CO-narcosis caused no glycosuria in a dog fed four consecutive days on bread only.

For such experiments suggest that sugar is formed during ether- or CO-narcosis but that it does not escape from the body after an exclusive

carbohydrate diet: and if this were so, some connection between carbohydrate diet and the activity of the pancreas would not be improbable.

Again it is obvious that since the pancreas is perhaps the most important of all the digestive glands, a state of digestion or fasting must greatly affect its activity: and so possibly the production of its internal secretion. And in this connection some injection experiments of Doyon and Dufourt[89] are very suggestive. They injected 2 gm. (per kilo of body-weight) of sugar into the saphenous vein of a dog, using a 1 in 6 solution. They usually took from 15 to 80 minutes to inject about 25 gm. of glucose: and in such a case about 3·5 gm. came out after fifteen minutes and only about ·35 gm. after 80 minutes. Their experiments were to prove that the rate of injection had a very important influence: but, as Blumenthal has shown, till the saturation limit of the blood is passed, the rate of injection has no perceptible influence. However they also tried the effect of injecting sugar into dogs kept without food for very long periods and they found that, if the sugar was injected at the same rate, the percentage amount consumed slightly diminished with a state of increasing fast. They remark: "It would seem that even in the fasting state the tissues of the animal are less ready to utilise glucose[90]."

Before describing my own experiments one or two explanatory notes are desirable:

1. In the Tables of Experiments the letters R.P. always stand for "Reducing Power reckoned as if due to glucose."

2. All my estimations have been made with Pavy's fluid, which I have found to give me accurate results when estimating sugar solutions of known composition. In many cases I have checked my results with a polarimeter and by fermentation.

3. The figures given as the R.P. of urine are in every case those obtained before inverting with acid. I am quite aware that Pavy showed that the R.P. of normal urine and of urine in cases of glycosuria could in nearly every case be increased by inverting with an acid; but as I omitted to invert the urines in my earlier experiments and in some of my later ones, I have thought it better throughout to give the figures obtained before inversion, especially as my results do not pretend to have more than a comparative value, or to be free from the errors associated with experiments on living animals in which injections are given.

4. The urine of all dogs has a certain R.P. due as Pavy and others have shown to the presence among other bodies of a certain amount of carbohydrate. In many of my experiments I have estimated the R.P. of the urine before the experiment and in these cases this is the figure I have usually deducted from the R.P. of the urine after morphine injection. But in cases where I have omitted to estimate the R.P. before the experiment or have been unable to get a sample of urine (as in nearly all experiments on male dogs), I have taken the figure for the R.P. of the urine before the experiment from the figures I obtained in 11 experiments on dogs A and B in which I found that the average R.P. of a normal dog's urine fed the evening before an experiment is about ·17 %.

In a footnote to each Table I have stated what allowance was made for the normal R.P. of the urine in calculating the quantity of excreted sugar in the urine.

5. In experiments on cats recorded in the second part of this paper, it frequently happened that I was unable to get a sample of urine before anæsthetising the animal: and that in comparison with the small quantity of urine secreted during the experiment the bladder contained a large amount of urine. In these experiments I have taken ·2 %, as representing approximately the average R.P. of a normal cat's urine.

My experiments on the behaviour of injected glucose were done on two dogs during the summer of 1905 in the pharmacological laboratory at Heidelberg,—I take this opportunity of expressing my gratitude to Prof. Gottlieb for his assistance and kindness in letting me work in his laboratory—and are summarised in Table I, but, as the intravenous injection of glucose was given while the animal was under the influence of morphine, some of the results were somewhat obscured by the glycosuria caused by the morphine-narcosis itself.

However in these experiments there is nothing to confirm the statement of Doyon and Dufourt that less sugar is consumed in a state of fast. Thus, if Exp. A 3 be compared with A 4 (*see* Table I) we see that in both ·25 gm. of glucose per kilo of body-weight was injected intravenously during the same interval of time, *i.e.* 5 minutes —and whereas 14 % of the sugar injected was excreted in A 3 in which experiment the dog had been fed the evening before: in A 4 only 7 % of the sugar injected was excreted, although the dog had fasted for 60 hours. And further in A 4 morphine had led to slight glycosuria, whereas in A 3 no glycosuria at all had occurred after morphine,

TABLE I. *Intravenous injection of glucose*[1] *in dogs under the influence of morphine.*

Exp.	Weight in kg.	State of alimentary canal. Time of last meal	Amount of glucose per kg. of body-weight injected	Quantity glucose excreted. Percentage quantity of glucose excreted	Periods of excretion	Remarks[2]
A 2 July 17th	12·5	Fasting — 36 hrs. before	·4 gm. (5 gm. in 10 % sol.)	1·723 gm. — 34·5 %	1·116 gm. in ½ hr., ·607 gm. in 1 hr., 0 in next hour	The injection was given 1 hr. 50 mins. after the morphine: in which time about ·155 gm. of sugar had been excreted.
A 3 July 25th	14·5	Digesting — Evening before	·25 gm. (3·62 gm. in 20 % sol.)	·514 gm. — 14 %	In 1 hr.	The injection was given 3½ hrs. after morphine: in which time no glycosuria had occurred.
A 4 Aug. 1st	13·6	Fasting — 60 hrs. before	·25 gm. (3·4 gm. in 20 % sol.)	·225 gm. — 7 %	In 1 hr.	The injection was given 3 hrs. after the morphine: in which time only ·06 gm. of sugar had been excreted.
B 3 July 21st	12	Digesting — Evening before	·3 gm. (3·6 gm. in 20 % sol.)	2·21 gm. — 61 %	1·93 gm. in 1 hr., ·28 gm. in 4½ hrs.	The injection was given 1½ hrs. after morphine: and part of the sugar excreted was certainly due to the effect of morphine.
B 4 July 28th	11·6	Fasting — 60 hrs. before	·3 gm. (3·48 gm. in 20 % sol.)	·756 gm. — 22 %	·756 gm. in 1 hr., 0 subsequently	The injection was given 3 hrs. after morphine: in which time only ·023 gm. of sugar was excreted so that nearly all the glycosuria was due to the injected sugar.
B 5 Aug. 14th	10·9	Fasting — 60 hrs. before	·3 gm. (3·27 gm. in 20 % sol.)	·293 gm. — 9 %	In 2½ hrs.	The injection was given about 4 hrs. after morphine (followed by the operative procedure of ligaturing the thoracic duct under ether) had led to a marked glycosuria, which was now passing away.

[1] The glucose was injected into the saphenous vein during five minutes.

[2] In calculating the quantity of sugar excreted in these Exps., the figure expressing the reducing power of the normal urine at the commencement of the experiment was subtracted from the figure expressing the reducing power of the sugar-containing urines.

although as will be shown in the second part of this paper the effect of morphine in causing glycosuria is beginning to pass off after 3 hours, which was the time which had elapsed in both these experiments when the injection of glucose was given. The effect of morphine is well shown in A 2 where the injection was given only 2 hours after the morphine and where the morphine certainly led to well-marked glycosuria. For here 34·5 % of the sugar injected was excreted, although at the same time it is to be remembered that the excretion of sugar following morphine-narcosis had only amounted to ·155 gm. in 1 hr. 50 mins.

The three experiments on dog B in each of which ·3 gm. of glucose per kilo of body-weight was injected intravenously during the course of 5 minutes, gave results somewhat obscured by the morphine effect: especially B 3 in which the injection of glucose was given only 1½ hours after the morphine, so that the very large amount of sugar excreted (viz. 61 % of the sugar injected) was certainly partly due to the morphine. However in B 5 the glycosuria caused by morphine was certainly passing away, and the result (9 % excreted after 60 hours' fast) is somewhat similar to that obtained in A 4 (7 % excreted after 60 hours' fast), the difference being easily covered by experimental error and the fact that in B 5 a bigger injection of glucose was given (viz. ·3 gm. per kilo as against ·25 gm. per kilo).

I also did a couple of experiments without morphine on the subcutaneous injection of sugar during fasting. The results obtained merely confirmed the previous work of Pavy[9] and Scott[10] who, among others, showed that after subcutaneous injection of sugar there is sufficient time for it gradually to diffuse throughout the body and be taken up by the liver before hyperglycæmia ever arises. Scott, for instance, injected 150 gm. to 200 gm. of sugar subcutaneously into a dog weighing 25 kilo, and yet only from ·5 gm. to ·8 gm. appeared in the urine.

These are all the experiments I did on dogs, because it was not convenient to give accurate injections of sugar without an anæsthetic, and the moment an anæsthetic is given the result is not so clear. Therefore the remaining experiments I made were done on myself without anæsthetics at Cambridge. My friend Mr Arthur Cooke was kind enough to put his surgical skill at my disposal and I here wish to express my gratitude to him for all the trouble he was put to in these experiments.

We found the only accurate way of injecting sugar was to make an incision about 1 to 2 inches long and to tie a cannula into the vein as

in an experiment on an animal. The syringe used was specially made and was calibrated by me with great care, so that the injections were given with as much accuracy as possible. The sugar solution used was made to contain 20 % of pure glucose (obtained from Kahlbaum). It was then filtered into a sterile flask and sterilised for three days running by being put in a steam incubator at nearly 100° C. for one hour each day. It was then used and the residue of sugar left was most carefully estimated both by Pavy's fluid and with the polarimeter, in order to obtain the strength of the sugar solution actually used. There was slight loss after sterilising.

The injections in all cases were made into one of the subcutaneous veins of the forearm, usually the median-basilic: and the interval of time during which the injection lasted was about fifteen minutes, but as the experiments were done in pairs, I was careful that the interval was just the same in each pair which I wished to contrast.

The results I obtained are summarised in Table II. It will be seen that no sugar at all was excreted in the first or preliminary experiment in which I injected only 4·35 gm. in all. In the next two experiments, in which ·25 gm. of glucose per kilo of body-weight was injected during digestion after a previous diet rich and extremely poor in carbohydrate respectively, it will be seen that the difference in the amount of sugar excreted in the two cases was not great enough to prove that there was any difference in the influence exerted by the two diets, though the result was suggestive.

I next tried if there was any difference between a diabetic diet containing but little carbohydrate and actual starvation for 60 hours. Owing to the fact that in Exps. 2 and 3 I got a very different quantity of urine excreted in the hour following the injection of sugar, I tried to get the body in the same state of fluid-content by having no food or drink on the morning of the experiments and by drinking on the previous three days approximately the same quantity of fluid. In this pair of experiments I injected approximately ·36 gm. of glucose per kilo of body-weight. It will be seen that the difference in the amount of sugar excreted in the two cases was not great enough to prove that there was any difference in the influence exerted by a state of pancreatic activity and rest respectively.

But as the quantity of urine, in spite of my precautions, secreted in the two cases was very different, I next tried two more experiments (Exps. 6 and 7) in which I drank nothing at all in the three days previous to the morning of the experiment and stimulated renal

TABLE II. *Intravenous injection of glucose in man (I. T.) during carbohydrate diet, proteid diet, hunger, and digestion.*

Number and date of exp.	Body-weight in kg. R. P. of urine before exp.	Previous diet in relation to carbohydrate	Time of last meal. Nature of last meal	Quantity of glucose injected per kg. of body-wt. Total quantity of sugar injected	Quantity of sugar excreted. Percentage quantity of sugar excreted
Exp. 1 Dec. 28, 1905	58·96 ·27 %	Ordinary (fat, proteid, carbohydrate)	Morning of exp. Rich in sugar	·073 gm. 4·35 gm.	0 0
Exp. 2 Jan. 4, 1906	58·51 ·36 %	Ordinary	Morning of exp. Rich in sugar	·25 gm. 14·628 gm.	·1633 gm. 1 %
Exp. 3 Jan. 19, 1906	59·2 ·15 %	Strict diabetic, minimum quantity of carbohydrate	Morning of exp. No carbohydrates	·25 gm. 14·8 gm.	·59 gm. 4 %

Exps. 2 and 3 were done to show the influence of diets rich and extremely poor in carbohydrates respectively on the consumption in the body of sugar injected during digestion.

Exp. 4 Mar. 22, 1906	56·24 ·19 %	Diabetic containing a little carbohydrate	Evening before Diabetic diet	·358 gm. 20·134 gm.	1·09 gm. 5·5 %
Exp. 5 Mar. 29, 1906	55·11 ·32 %	Strict diabetic plus 60 hrs. starvation	60 hrs. before	·363 gm. 20·05 gm.	·867 gm. 4 %

Exps. 4 and 5 were done to show the influence of rest of the alimentary canal, after a diet practically free from carbohydrate, on the consumption of sugar in the body.

In these two experiments no fluid was drunk on morning of experiment: and approximately the same quantity of fluid on each of the 3 previous days.

Exp. 6 June 14, 1906	57·7 ?,	Ordinary diet followed by 2 days free from carbohydrate	Evening before No carbohydrate	·436 gm. 25·155 gm.	1·88 gm. 7·5 %
Exp. 7 June 21, 1906	55·34 ·35 %	Diet free from carbohydrate plus 60 hrs. starvation	60 hrs. before	·436 gm. 24·141 gm.	1·27 gm. 5·25 %

In Exps. 6 and 7 no fluid was drunk in the 60 hrs. preceding the experiment: but on the morning of the experiment renal activity was stimulated by taking ·3 gm. of caffeine-hydrochloride.

These two experiments were done with the same object as the last two: but in these two the alimentary canal was in an even greater state of rest as no food or *drink* was taken for 60 hrs.

Note:—In calculating the quantity of sugar excreted in the urine, allowance was made for the R.P. of urine before the experiment.

activity on the morning of the experiment by taking ·3 gm. of caffeine-hydrochloride dissolved in 50 c.c. of water[1]. From these, in each of which ·463 gm. of glucose per kilo was injected after a previous diet poor in carbohydrate and after a diet containing no carbohydrate at all followed by 60 hours of starvation respectively, it will be seen that the difference in the amount of sugar excreted is not great enough to prove that there is any difference in the influence exerted by a state of activity and rest respectively of the alimentary canal (including the digestive functions of the pancreas). For in the last experiment nothing was taken by the mouth for 60 hours before the morning of the experiment[2].

The chief result, then, which may be considered as proved by these experiments, is, that the state of the alimentary canal, including the pancreas, as regards activity or rest has no influence on the consumption of sugar intravenously injected into the body. And consequently we may make the deduction that the internal secretion of the pancreas is not affected by its digestive activity or rest, and that Pflüger's assertion (33) that the pancreas has no internal secretion but produces its effect through its digestive functions alone must be rejected. But these experiments do not afford any evidence that normally there is no variation in the internal secretion of the pancreas.

[1] A preliminary experiment with caffeine-hydrochloride showed that it had a marked effect in producing diuresis: as shown by the following experiment:

May 28, 1906—Took fluid all day freely.
,, 29, ,, —Eat food but took no fluid.
,, 30, ,, — ,, ,,
,, ,, ,, 7.15 p.m. } 67 c.c. urine secreted at rate of 27 c.c. per hr.
,, ,, ,, 9.45 p.m. }
,, 31, ,, 5.15 a.m. } 211 ,, ,, ,, 28 ,, ,,
,, ,, ,, 5.15 a.m. Drank ·3 gm. caffeine-hydrochloride dissolved in 100 c.c. water.
,, ,, ,, 5.15 a.m. }
,, ,, ,, 5.45 a.m. } 23 c.c. urine secreted at rate of 46 c.c. per hr.
,, ,, ,, 6.45 a.m. } 53 ,, ,, ,, 53 ,, ,,
,, ,, ,, 7.45 a.m. } 53 ,, ,, ,, 53 ,, ,,
,, ,, ,, 8.45 a.m. } 40 ,, ,, ,, 40 ,, ,,

[2] I again failed to obtain the same quantity of urine secreted in the two experiments from which it may be gathered that it is very difficult to obtain the same state of renal activity and the same quantity of fluid in the body on two different occasions in spite of such precautions as drinking no fluid for 60 hours previous to the experiment and being careful not to take exercise likely to cause perspiration.

As regards the experiments of Doyon and Dufourt, I think that in view of my own results it may be considered that the difference which they found between the amount of sugar excreted after intravenous injection into normal and fasting dogs was probably the result of experimental error, especially as the difference was distinctly small in amount: although there is no doubt, as Hofmeister's experiments proved, that alimentary glycosuria is more easily produced in a fasting animal[23].

Before leaving this subject it is interesting to notice that Falta[13] (working with others) has lately put forward a view of "Diabetes Mellitus," according to which the disease would consist in a disturbance of the balance between the internal secretion of the pancreas and certain other internal secretions, notably those of the supra-renal capsule and thyroid[13]. There is much truth in what he says, but he bases a good deal on the hypothesis that proteid in the diet in some way stimulates the thyroid to increased secretion, while carbohydrate has a like effect on the internal secretion of the pancreas. This hypothesis, as regards the pancreas at least, is not disproved by my experiments, as in Exps. 3 and 4, the only pair in which I compared the effect of a markedly carbohydrate diet with a strict diabetic diet; the difference in the excretion of injected sugar was all in favour of the experiment associated with carbohydrate diet. The result, however, is far from conclusive, as the reducing power of the urine beforehand in the two experiments was very different.

I now proceed to consider, in the second part of this paper, the mechanism of morphine and ether-glycosuria, and again we shall see that glycosuria associated with narcosis is uninfluenced by the digestive activity of the pancreas.

Part II.

As early as 1899 I did some experiments, in which I found that glycosuria associated with morphine-narcosis in dogs occurred apparently more readily in a state of fast than after a carbohydrate or mixed diet; but in the past ten years numerous experiments have shown me that the difference between a state of fasting and digestion is not as clear as I at first thought, and I am now of opinion that a state of digestion is, if anything, more likely to be attended by glycosuria.

My earlier experiments on morphine-glycosuria gave no clear result because they were done on different individuals, but since I have

TABLE III. *Effect of morphine in causing glycosuria in various states of digestion and fasting after a mixed diet.*

Number of exp.	Weight in kg.	State of alimentary canal. Time of last meal	Quantity of morphine given per kg.	Quantity of sugar excreted	Period of excretion	Remarks
A 1 July 6	13·2	Digesting —— Evening before	·0053 gm. subcut.	0 ? plus	In 22 hrs.	The perineum was split in this exp. The R.P. of the urine was only ·175 % but as the quantity of urine was large it is possible that ·215 gm. was excreted.
A 2 July 25	12·5	Fasting —— 36 hrs. before	·006 gm. subcut.	·155 gm. ? plus	In 1½ hrs.	The amount of sugar excreted is only given approximately as some urine was lost. Some glycosuria certainly occurred as the R.P. of urine was raised from ·17 % to ·62 %.
A 3 July 25	14·5	Digesting —— Evening before	·007 gm. subcut.	0	In 3 hrs.	The R.P. of urine 3 hrs. after the morphine was actually lower than before: and the R.P. of urine was not raised by boiling with acid.
A 4 Aug. 1	13·6	Fasting —— 60 hrs. before	·007 gm. subcut.	·03 gm.	In 2¾ hrs.	*Vide* Table I. The amount of sugar excreted subsequently after the injection of sugar was extremely small implying that the morphine had very little more effect in causing glycosuria.
A 5 Aug. 8	14·0	Fasting —— 60 hrs. before	·007 gm. intraperit. also later ether for 1½ hrs. and operation	·028 gm. in 1½ hrs. 1·26 ,, ,, 1½ ,, ·163 ,, ,, 1½ ,, ·06 ,, ,, 2½ ,, 1·511 ,, ,, 7½ ,,		The ether was given 1½ hrs. after the morphine: and the effect of the operation of ligaturing the left subclavian and innominate veins is at once seen: the urine of the first period after giving ether having the high R.P. of 7·2 %.
B 1 July 4	12·0	Digesting —— Evening before	·005 gm. subcut.	4·05 gm.	In 24 hrs.	The perineum was split in this exp.
B 2 July 10	11·6	Fasting —— 36 hrs. before	·00435 gm. intraperit.	·23 gm.	In 7½ hrs.	——

TABLE III. (*Continued.*)

Number of exp.	Weight in kg.	State of alimentary canal. Time of last meal	Quantity of morphine given per kg.	Quantity of sugar excreted	Period of excretion	Remarks
B 3 July 31	12·0	Digesting —— Evening before	·007 gm.	·057 gm. ? plus	In 1½ hrs.	Subsequently after injecting sugar into the saphenous vein, more sugar was excreted which was certainly partly due to the morphine but did not exceed 2 gm.
B 4 July 28	11·6	Fasting —— 60 hrs. before	·007 gm. subcut.	·023 gm. ? plus	In 3 hrs.	Subsequently after injecting sugar into the saphenous vein, more sugar was excreted which may have been partly due to the morphine, but did not exceed ·818 gm.
B 5 Aug. 4	10·9	Fasting —— 60 hrs. before	·007 gm. intraperit.	2·1 gm. ? plus	In 4 hrs.	This sugar was excreted under the stimulus not only of morphine but also of a very little ether and the operation of ligaturing the left subclavian and innominate veins. The urine had the high R.P. of 6·5 %. Subsequently after injecting sugar, more sugar was excreted which did not exceed ·38 gm.

In calculating the quantity of sugar excreted in these experiments the figure expressing the R.P. of the normal urine at the commencement of the experiment was subtracted from the figure expressing the R.P. of the sugar-containing urines.

undertaken a series of experiments on the same individual, I have obtained some results, which, though not very striking, are distinctly interesting.

I have experimented, whenever it was possible, with bitches, because in these it is far easier than in dogs to catheterise the bladder and draw off the urine as desired. In my later experiments I used morphine tartrate for injection, because it is more soluble than the hydrochloride salt, but the effect seems to be identical.

The first experiments on the relation of morphine-narcosis to digestion and starvation, which I propose to describe, are ten done on dogs A and B at Heidelberg in 1905. Both dogs were fed every evening about 7 p.m. on scraps from restaurants; mostly meat, but

also some bread, etc. On the days when the dog fasted it was given water to drink.

B was a mongrel type of large terrier; fat and in good condition, over five years of age from the state of its teeth. It was of an unexcitable temperament and slept very readily under the influence of morphine which rapidly produced its usual effects.

A was a mongrel type of sheep-dog; not more than two years old, in good condition but of a rather nervous temperament, so that it did not sleep nearly as readily as B under morphine.

From Table III, summarising these ten experiments, it will be seen that in dog B morphine led to the excretion of sugar better during digestion than starvation, but that a considerable excretion of sugar took place after 60 hours' fast, when the morphine was supplemented by the operation for establishing a fistula of the thoracic duct, in which considerable stimulation of nerves must have occurred.

In dog A just as morphine had not a ready effect in causing profound sleep, so neither after digestion nor after fasting did it lead to the excretion of much sugar. But, as in B the added effect of the operation for establishing a fistula of the thoracic duct (under ether in this case) led to the excretion of a considerable amount of sugar.

These experiments appear to have given clearer results than was the case in some 15 experiments I did in 1901—1902 on four dogs, a summary of which is given in Table IV.

It will be seen that more sugar was excreted after a day's fast than during digestion in dogs C and D though not in E. But the most noticeable feature about these experiments is the variability. Thus D 1 and D 3 were both done during digestion: yet in the first, ·074 gm. only as against 1·94 gm. in the second was excreted. The same is seen in comparing E 1 with E 2. In those cases where little sugar was excreted—as in D 1 and E 1—I cannot help thinking that splitting the perineum may have had a reflex nervous influence on the renal mechanism, resulting in a diminished excretion of sugar which was possibly present in the blood.

Again the difference between individuals in their reaction to morphine is well seen in a series of 12 experiments done on four dogs in 1905—1906, which are recorded in Table V.

From these it will be seen that whereas in J morphine always led to a considerable excretion of sugar, in I it only led to a slight excretion.

The six experiments on I given also show the variability in the amount of sugar excreted by a dog under morphine, where the last

TABLE IV. *Effect of anæsthetisation with ·007 gm. of morphine tartrate per kg. of body-weight (with and without splitting of the perineum under A.C.E.), in causing glycosuria in dogs which were given the morphine subcutaneously soon after a meal or after a day's fast, the dogs having been fed on meat or meat and biscuits.*

Number of exp.	Weight in kg.	Digesting or Fasting	Remarks	Periods of excretion of sugar	Total quantity of sugar excreted. Highest percentage of sugar in urine
C 1 Sept. 3	6·2	20 hrs. after food	Perineum split under A.C.E.	·059 gm. in ½ hr. ·874 ,, 1 ,, ·117 ,, 1½ ,, ·019 ,, 2 ,,	1·06 gm. in 5 hrs. —— 8·9 %
C 2 Sept. 10	6·3	Digesting	——	·149 gm. in ½ hr. ·809 ,, 1 ,, ·656 ,, 1½ ,, ·037 ,, 3 ,,	1·651 gm. in 6½ hrs. —— 6·5 %
C 3 Sept. 25	6·12	Day's fast	——	·420 gm. in 1 hr. 1·309 ,, 1 ,, ·779 ,, 1 ,, ·513 ,, 2 ,, ·073 ,, 3 ,,	3·094 gm. in 8 hrs. —— 12·2 %
D 1 Sept. 6	7·1	Digesting	Perineum split under A.C.E.	·003 gm. in ½ hr. ·071 ,, 3½ ,,	·074 gm. in 4½ hrs. —— ·6 %
D 2 Sept. 27	7·75	Day's fast	——	·0132 gm. in ½ hr. ·0358 ,, 2 ,, 1·386 ,, 1½ ,, 1·984 ,, 4½ ,,	3·4198 gm. in 7½ hrs. —— 10·2 %
D 3 Nov. 11	7·85	Digesting	——	·008 gm. in 1 hr. 1·463 ,, 2½ ,, ·468 ,, 3½ ,,	1·94 gm. in 7 hrs. —— 8·3 %
D 4 Dec. 2	7·35	Day's fast	——	·012 gm. in 1 hr. ·792 ,, 2½ ,, ·56 ,, 3½ ,,	1·364 gm. in 7½ hrs. —— 7·5 %
D 5 Jan. 28	8·25	18 hrs. after food	——	·042 gm. in 2½ hr. ·649 ,, 20½ ,,	·692 gm. in 22¾ hrs. —— 1 %
D 6 Feb. 21	7·75	Day's fast	——	·66 gm. in 6½ hr. ·21 ,, 16½ ,,	·87 gm. in 23 hrs. —— 3·8 %
E 1 Oct. 1	9·1	Digesting	Perineum split under A.C.E.	·0113 gm. in 1 hr. ·1018 ,, 1 ,, ·0366 ,, 1½ ,, ·0505 ,, 2 ,,	·18 gm. in 5½ hrs. —— 1·1 %
E 2 Oct. 14	9·1	Digesting	——	·1814 gm. in 1½ hr. 1·6978 ,, 2½ ,, ·0737 ,, 3 ,,	1·953 gm. in 6½ hrs. —— 4·5 %
E 3 Nov. 4	9·4	Day's fast	——	·0107 gm. in ½ hr. ·24 ,, 1 ,, ·51 ,, 1½ ,, ·0384 ,, 1¾ ,,	·799 gm. in 4½ hrs. —— 6·3 %
E 4 Nov. 25	9·3	18 hrs. after food	——	·102 gm. in 1½ hr. ·799 ,, 2½ ,, ·007 ,, 5 ,,	·908 gm. in 8½ hrs. —— 6·8 %
E 5 Dec. 4	8·9	Day's fast	——	·0903 gm. in 1½ hr. ·48 ,, 2 ,, ·016 ,, 1½ ,,	·586 gm. in 5½ hrs. —— 4·3 %
F	8·05	Digesting	Perineum split under A.C.E.	·130 gm. in 2½ hr. 1·705 ,, 8½ ,, ·128 ,, 5½ ,,	1·963 gm. in 11½ hrs. —— 5·5 %

In calculating the quantity of sugar excreted allowance was made for the R.P. of urine before the experiment.

TABLE V. *Effect of anæsthetisation with morphine tartrate or ether alone; or with morphine succeeded by ether (plus some operative procedure in 3 cases) in causing glycosuria in dogs which had been kept for at least a week previously on a pure meat diet (except in Exp. I 4), and which had been fed the day before the experiment.*

Date and number of exp.	Weight in kg.	Anæsthetic placed opposite the period of its effect	Periods of excretion	Total quantity of sugar excreted	Remarks
G	12·5	·007 gm. M.T. per kg. Ether 15 mins. ...	·001 gm. in 1 hr. ·718 ,, 3½ ,, ·014 gm. later	·72 gm. in 24 hrs.	The administration of ether was not followed by any rise in R.P. above previous level. The perineum was split in this exp.
H	16·75	·012 gm. M.T. per kg. This dog was also pithed. Ether ½ hr. ...	2·8 gm. in 4½ hrs. ·45 gm. in 1 hr.	3·25 gm. in 5½ hrs.	The R.P. of last urine estimated was 3·6 %: so more sugar was subsequently excreted. The ether was followed by a slight rise in R.P.
I 2 Oct. 25	13·25	·01 gm. M.T. per kg.	·94 gm. in 3½ hrs.	——	More sugar was certainly excreted subsequently: as R.P. of last urine estimated was 3·8 %.
I 3 Nov. 1	12·8	Ether 20 mins. ...	·054 gm. in 2 hrs.	——	R.P. of last urine estimated was ·71 %.
I 4 Jan. 26	13·3	·01 gm. M.T. per kg. Ether 1 hr.	·085 gm. in 3 hrs. ·27 gm. later	·35 gm. in 24 hrs.	The diet during past two months has been biscuits as well as meat.
I 5 Feb. 7	12·0	·01 gm. M.T. per kg. Ether 1 hr. ...	·12 gm. in 2 hrs. ·038 ,, 2 ,, ·021 ,, 2½ ,,	·18 gm. in 6½ hrs.	R.P. of last urine estimated was only ·32 %.
I 6 Feb. 21	11·75	Ether ¾ hr. ...	·088 gm. in 3 hrs. ·046 ,, 3 ,,	·13 gm. in 6 hrs.	R.P. of last urine estimated was only ·41 %.
I 7 June 19	10·75	Chloroform, A.C.E., and ether	·45 gm. in 2 hrs. ·38 gm. later	·83 gm. in 24 hrs.	A fistula of the thoracic duct was established in this exp.
J 1 Nov. 1	8·45	Ether 20 mins. ...	·029 gm. in 1½ hrs.	——	R.P. of last urine estimated was ·5 %.
J 2 Nov. 8	8·9	·009 gm. M.T. per kg. Ether 1 hr. ...	2·052 gm. in 2 hrs. 1·88 ,, 1½ ,,	3·93 gm. in 3½ hrs.	The ether was followed by a considerable rise in R.P., the last urine estimated had the R.P. of 9·6 %: so more sugar was certainly excreted subsequently.
J 3 Nov. 15	7·75	·01 gm. M.T. per kg. Ether ½ hr. ...	·76 gm. in 1½ hrs. 1·9 ,, 2 ,,	2·66 gm. in 2½ hrs.	The R.P. of last urine was 6·5 %. This urine was passed so soon after the morphine that the rise in R.P. may have been due as much to the morphine as ether.
J 4 Nov. 22	6·89	·01 gm. M.T. per kg. Ether 1 hr. ...	1·66 gm. in 1½ hrs. ·51 ,, 2 ,,	2·17 gm. in 3½ hrs.	More sugar was certainly excreted subsequently as R.P. of last urine was 4·1 %. The ether was followed by a distinct drop in R.P.

In calculating the quantity of sugar excreted, allowance was made for the R.P. of the urine before the experiment.

food given was in all cases about the same time, namely at or before 4 p.m. on the day before the experiment.

Here we see that most sugar was excreted in the first of the series (I 2) and that in the last (I 7)—although the animal was considerably lighter in weight than in any of the others and so presumably the liver contained less glycogen—nearly as much sugar was excreted, viz., ·83 gm. under the added stimulus of the operation for making a fistula of the thoracic duct. This recalls a similar result in Exps. A 5 and B 5.

This series also gives me the opportunity for comparing the effect of ether and morphine; and I think it will be clear that morphine has, if anything, a more marked effect than ether in causing glycosuria. J 2 is the only experiment in which there was a marked rise in the R.P. of urine which might be due to ether. And in J 4 the ether was followed by a distinct drop in R.P.

Again comparing the effect of ether alone as against morphine alone I 6, and I 5, and I 2 are interesting experiments. For we see that giving ether for ¾ hour led to the excretion of ·13 gm. in six hrs., while ·01 gm. of morphine tartrate per kilo of body-weight led to the excretion of ·15 gm. in four hours in I 5 and ·94 gm. in 3¾ hours in I 2. Also in J 1 ether alone produced but slight glycosuria although J was a dog in which morphine caused glycosuria readily.

Seelig was the first to show[10], as far as I know, that ether produces a distinct, though small glycosuria in meat-fed dogs, and he apparently considered that the glycosuria of ether-narcosis was due to deficient oxidation of sugar produced by proteid metabolism. For he said (1) that ether-narcosis causes no glycosuria in dogs fed for some time on carbohydrate; (2) that oxygen-infusion has no influence on a glycosuria already caused by ether, but that infusion of oxygen, at the same time as ether is given, prevents glycosuria.

He supposed that a carbohydrate diet protected the proteid metabolism from which the sugar arose and that in the absence of circulating proteid there was no glycosuria; while from his experiments on oxygen-infusion I gather that he considered the accumulation of sugar in the blood was bound up with deficient oxidation.

As a criticism of Seelig's paper: I would point out that the connection he seeks to establish between oxygen-infusion and ether-glycosuria is not supported by his own experiments; for on p. 489 in a footnote an experiment is described in which ether was given at 11 a.m. and also oxygen-infusion at the same time, and yet we read "The infusion is continued; after 1½ hours sudden death; the urine contains

sugar throughout." Also on p. 489 are described two experiments in which glycosuria followed oxygen-infusion; and this failure of oxygen-infusion to stop glycosuria he attributes to too much oxygen having been given, although he never proves by a series of experiments that too much oxygen has this effect. Lastly he admits that a glycosuria already existing cannot be stopped by oxygen-infusion but gets greater than otherwise, as shown for instance by the experiment of Nov. 2, 1903

Therefore I do not think that Seelig's own experiments afford much proof that oxygen-infusion at the same time as ether-narcosis prevents glycosuria. On the other hand his statement about the relation of carbohydrate diet to ether-narcosis, supported by the fact that Straub also found [7], that glycosuria after CO-inhalation did not occur in dogs fed on carbohydrate, is so important, that I have lately undertaken a fresh series of experiments to test this point. For as I have already explained in the first part of this paper my reading of such a connection, if true, would be that it pointed to some alteration in the amount of the pancreas's internal secretion, because I imagine that ether and morphine-narcosis invariably produce some conversion of hepatic glycogen into sugar; and so, if after a carbohydrate diet glycosuria does not occur for some reason, I should consider that the most probable explanation of this would be bound up with variations in the activity of the pancreas. But it is not true.

As will be seen from the experiments (Table VI), in which I tested the effect of a carbohydrate diet on the glycosuria of both morphine and ether-narcosis, I have failed entirely to confirm Seelig's statement.

Table VI embodies some experiments which I did in 1909 at Stockholm through the courtesy of Professor Johansson, whose kindness I am glad to have this opportunity of mentioning. I performed seven experiments on three dogs all fed on a carbohydrate diet, consisting of Spratt's puppy biscuits till (in most cases) a day or two before the experiment when only bread and Marie biscuits were given; and although I was not successful in obtaining in every experiment pronounced glycosuria as the result of ether-narcosis, yet I obtained a very definite effect in M 1 and a less definite effect in M 2, K 2 and K 3. At the same time this Table proves how much more effective morphine is than ether in causing glycosuria, as shown by comparing L 1 with L 2, and K 1 with K 2 and K 3.

These experiments also showed, like the experiments recorded in the previous Tables: (α) great individual difference in the readiness with which anæsthetic glycosuria could be produced (thus L was an

TABLE VI. *Effect of carbohydrate diet on morphine and ether glycosuria.*

[The ether in these Exps. was given for 1 hr.: and a subcutaneous injection of ·01 gm. of morphine tartrate per kilo of dog's weight was in each case the dose given.]

Number of exp.	Nature of food previous to exp. Weight on morning of exp.	R.P. of urine at beginning of exp.	Experimental procedure	Quantities of urine and sugar excreted during course of exp.	R.P. of urine on morning of day following exp.
K 1 Sept. 20	I had this dog in lab. for 3 days before exp. Spratt's puppy biscuits and water only food given. 15·7 kilos.	—	Morphine. Perineum slit 1 hr. after morphine	135 c.c. R.P. 1·11 %=1·215 gm. in 1 hr. 27 c.c. R.P. 10·1 %=2·727 gm. in 1 hr. 8 c.c. R.P. 5·12 %= ·409 gm. in 1 hr. Total 4·35 gm. in 3 hrs.	—
K 2 Oct. 5	Fed on meat till 3 days before exp. when it was put in a cage and fed for 2 days on Spratt's puppy biscuits and 1 day on bread and Marie biscuits of which it eat more than 500 gm. 14·7 kilos.	10 mins. after start. 1 % [1·83 %]	Ether. Interval of 40 mins. Morphine ...	1·5 c.c. R.P. 1·6 %= ·024 gm. in 55 mins. 6 c.c. R.P. ·72 %= ·043 gm. in 1 hr. [1·4 %] 12 c.c. R.P. 3·4 %= ·408 gm. in 1 hr.* [3·79 %] 18·5 c.c. R.P. 3·1 %= ·573 gm. in 1 hr.* [3·33 %] Total 1·048 gm. in 3 h. 55 m.	At 11 a.m. 280 c.c. R.P. ·157 % [·33 %]
K 3 Oct. 18	Fed for 11 days on Spratt's puppy biscuits, with 4 oz. of meat on 8 occasions. During 48 hrs. before exp. was put in cage and fed only on bread and Marie biscuits, of which it eat more than 500 gm. 14·3 kilos.	·61 % [1·1 %]	Ether. Interval of 5 mins. Morphine ...	1·4 c.c. R.P. 1·08 %= ·015 gm. in 1 hr. 6·5 c.c. R.P. ·87 %= ·056 gm. in 1 hr. 5·9 c.c. R.P. ·72 %= ·042 gm. in 1 hr. [1·81 %] 12 c.c. R.P. ·37 %= ·044 gm. in 1 hr. [·81 %] Total ·167 gm. in 4 hrs.	At 11 a.m. 185 c.c. ·29 %
L 1 Sept. 23	Fed for 5 days on Spratt's puppy biscuits and then for 2 days on bread only. 10·5 kilos.	—	Ether. (Perineum slit 20 mins. after commencement.) Interval of 40 mins. Morphine ...	5 c.c. R.P. less than ·4 %= ·02 gm. in 1 hr. 8 c.c. R.P. less than ·5 %= ·04 gm. in 1 hr. 12 c.c. R.P. ·35 % [·62 %] = ·042 gm. in 1 hr. 16·5 c.c. R.P. ·47 % = ·077 gm. in 1 hr. Total ·179 gm. in 4 hrs.	At 11 a.m. ·25 %

L 2 Oct. 11	Fed for a fortnight on Spratt's puppy biscuits and an occasional piece of meat. During 48 hrs. before exp. kept in cage and fed on bread and Marie biscuits, of which it eat about 700 gm. 10·4 kilos.	·27 % [·44 %]	Morphine ...	7 c.c. R.P. ·36 % = ·025 gm. in 1 hr. 17 c.c. R.P. 2·05 % = ·348 gm. in 1 hr.* 14·5 c.c. R.P. 1·91 % = ·276 gm. in 1 hr.* [2·1 %] Total ·649 gm. in 3 hrs.	—
M 1 Sept. 28	I had this dog in the lab. for 1 week before exp., fed all the time on Spratt's puppy biscuits only. 7·5 kilos.	25 mins. after commencement. ·13 % [·38 %]	Ether. (Perineum slit 15 mins. after commencement.) Interval of 30 mins. Morphine ...	3 c.c. R.P. 5·4 % = ·162 gm. in 1 hr.* [5·4 %] 8 c.c. R.P. 2·5 % = ·2 gm. in 1½ hrs.* [2·93 %] 6 c.c. R.P. 2·2 % = ·132 gm. in 1 hr. 10 m. [2·8 %] 6·75 c.c. R.P. ·27 % = ·018 gm. in 1 hr. Total ·512 gm. in 4 h. 25 m.	At 10 a.m. ·37 %
M 2 Oct. 14	Fed since last exp. on Spratt's biscuits only. During 24 hrs. before exp. it had bread only. 7·2 kilos.	·26 %	Ether. Interval of 45 mins. Morphine ...	3·5 c.c. R.P. 1·51 % = ·052 gm. in 1 hr. [2·08 %] 2·3 c.c. R.P. 1·2 % = ·027 gm. in 1 hr. 5 c.c. R.P. 3·27 % = ·163 gm. in 1 hr. [3·28 %] 7 c.c. R.P. ·76 % = ·053 gm. in 1 hr. Total ·295 gm. in 4 hrs.	At 11 a.m. ·68 %

All urines obtained with catheter.

All figures in brackets indicate reducing power of urine after inversion with HCl for 1 hr.

Quantity of sugar excreted calculated from R.P. of urine before inversion and no allowance is made in this Table for the normal R.P. of urine except in the first urine of K 1, where ·2 % has been deducted.

* indicates urines in which the presence of sugar was confirmed by fermentation with yeast for 3 hrs. at 37° C.

† indicates urines in which there was shown to be no fermentable sugar by the yeast test.

excitable type of lurcher-mongrel in good condition, but by no means fat; while K was a placid greedy type of mongrel, distinctly fat); (*b*) a tendency for the degree of glycosuria to get less with each experiment, associated with loss of weight produced by the cramped conditions of laboratory life.

Consequently I have no doubt that Seelig's statement is erroneous from being based on experiments done on a few individual dogs in which the glycogenic contents of the liver was small in amount. Similarly I do not think it surprising that Straub failed to get glycosuria in a dog fed on carbohydrate, if all his experiments were like that recorded on p. 156 of his paper. In this experiment a dog was fed on meat only on Dec. 3rd, 4th, 5th, and 6th and on 200 gm. of bread only each evening of Dec. 7th, 8th, 9th and 10th. Then CO-poisoning produced a very marked glycosuria on Dec. 7th, that is after four days of pure meat diet, on the 8th a distinct glycosuria, on the 9th slight glycosuria, and on the 10th no glycosuria at all. Now by the 10th the hepatic glycogen must have been small in amount after CO-poisoning on three previous days and a diet of only 200 gm. of bread daily; and so it is not surprising to my mind that no glycosuria occurred on the 10th.

As regards the mechanism of CO-glycosuria I see no reason for doubting that it is very similar to that of the glycosuria produced by anæsthetics and in particular by morphine. For Seelig failed to obtain glycosuria after CO-infusion, and Marcacci[40] and Mischka[39] similarly did not succeed in producing glycosuria with intraperitoneal or subcutaneous injections of CO. Therefore it looks as if CO-inhalations cause glycosuria by an indirect (reflex from the lungs) or direct asphyxial stimulation of certain centres in the central nervous system.

The source of the sugar excreted in morphine-glycosuria. The first point to note is that there is hyperglycæmia—just as Seelig found in ether-glycosuria—as shown for example by Exp. X (Table VIII), made in 1901, where the R.P. of the blood 2½ hours after giving morphine was ·259 %, at a time when considerable glycosuria existed. In Exp. O 3 (Table VII), also, the R.P. of the blood was ·26 % 4½ hours after injecting morphine.

Now as there is hyperglycæmia, we must first consider the question whether sugar accumulates in the blood because the pancreas fails to act under the influence of morphine—and so a state resembling pancreatic diabetes arises—or because sugar is thrown into the blood by the liver or other organs of the body.

That it has nothing to do with the pancreas may be regarded as certain, I think, for the following reasons:

(1) If the pancreas was inhibited by morphine from supplying its internal secretion to the body, it is not clear why there is less tendency for sugar to be excreted in the later experiments of a series on the same animal.

(2) The effect of a diminished quantity of the pancreas's internal secretion during morphine-narcosis is clearly seen in the following six experiments. (Table VII.)

In these three dogs the urine was in a state just bordering on glycosuria owing to the greater part of the pancreas having been excised: but in every case enough pancreas had been left behind either in the peritoneal cavity or grafted under the abdominal skin to prevent any marked glycosuria.

If now morphine had the effect of inhibiting the pancreas, it would inhibit the whole gland as effectually as a portion of it: but whereas morphine occasionally leads to the excretion of no sugar at all in a normal dog—as in A 3—or quite often to the excretion of very little sugar as in the six experiments on I (*see* Table V), here in all these six experiments morphine led to a most marked excretion of sugar, amounting in the various cases to two or three times the average quantity excreted by normal dogs in my best experiments.

Evidently the sugar accumulated in the blood because of a production of sugar in the body and not because of a sudden inhibition of the pancreas.

Again whereas a normal dog can be given a large quantity of sugar by the mouth and very little come out in the urine, as Hofmeister[68] and other experimenters have shown and I have found myself—for instance 50 gm. of glucose dissolved in water given by a tube in the œsophagus to a normal fasting dog led to the excretion of less than 1 gm. of sugar—in these dogs the sugar-consuming capacity of the body was much lowered. Thus in N 25 gm. of glucose were given by the mouth and half was excreted in the urine: and in O of 40 gm. given by the mouth 15 gm. were excreted. This shows that in these dogs the sugar-consuming capacity of the body was much lowered, as a very small amount of sugar entering the circulation could lead to glycosuria. *Where then does the sugar come from in morphine-glycosuria?*

As there is no evidence that the glycosuria is due to a diminished activity of the pancreas leading to a diminished consumption of sugar, it may readily be due to a sudden production of sugar in the body.

Now as the liver is a store-house of glycogen it is extremely probable that the sugar comes from the glycogen of the liver. This supposition is strengthened by the following considerations:

1. The glycosuria of morphine-narcosis is very like that of diabetic puncture. In both the glycosuria appears rapidly and has passed away in three or four hours. Also, as Claude Bernard, Luchsinger[17] and others showed, diabetic puncture does not produce glycosuria, if the

TABLE VII. *Effect of a subcutaneous injection of ·007 gm. of morphine tartrate per kilo of body-weight in causing glycosuria in dogs (fed on meat) during digestion. In each the pancreas had been excised and glycosuria averted by a piece of the gland either being left behind in the peritoneal cavity or grafted under the abdominal skin.**

Number of exp.	Weight in kg.	Remarks	Periods of excretion	Quantity of sugar excreted
C 4 Nov. 18	5·55	This dog's tendency to glycosuria was small as R.P. of urine before exp. was only ·22 %.	·9782 gm. in 1 hr. 2·736 gm. in 2½ hrs. ·981 gm. in 4½ hrs.	4·695 gm. in 8 hrs.
C 5 Dec. 5	5·9	The tendency to glycosuria now a little more, R.P. before exp. was ·33 %.	4·4239 gm. in 2½ hr. 3·2887 gm. in 4½ hrs. ·0308 gm. in 4 hrs.	7·743 gm. in 10½ hrs.
N	6·25	Tendency to glycosuria considerable, as R.P. of urine was about ·5 % and of 25 gm. of glucose given by mouth on this day, as much as 12·45 gm. were excreted.	3·08 gm. in 1½ hrs. 6·469 gm. in 4 hrs. ·323 gm. in 11½ hrs.	9·87 gm. in 17½ hrs.
O 1 Aug. 21	8·5	Perineum split under A.C.E. Tendency to glycosuria not great as R.P. of urine apart from exp. was only ·17 %.	2·556 gm. in 1½ hrs. 3·249 gm. in 1½ hrs. 1·362 gm. in 1½ hrs. ·097 gm. in 2½ hrs.	7·266 gm. in 6½ hrs.
O 2 Aug. 31	7·9	The sugar-consuming capacity of this dog may be gathered from two exps. done on Sept. 12 and 16 respectively when 40 gm. in solution were given it by the mouth and on each occasion about 15 gm. were excreted.	·005 gm. in ½ hr. 2·123 gm. in ½ hr. 3·886 gm. in 1 hr. 2·418 gm. in 1½ hrs. ·629 gm. in 2 hrs.	9·057 gm. in 5½ hrs.
O 3 Sept. 24	8·7	In this exp. the quantity of sugar excreted was not a fair measure of the morphine effect, as after 1½ hrs. 80 gms of blood were taken from the carotid for estimation of sugar therein. The R.P. of blood was ·26 %.	·86 gm. in ½ hr. 2·09 gm. in ½ hr. 2·46 gm. in 1 hr. 2·06 gm. in 2 hrs.	7·47 gm. in 4½ hrs.

In calculating the quantity of excreted sugar, allowance was made for the R.P. of urine before the experiment.

* In C and N a small piece of the pancreas retaining its own blood-supply had been grafted under the abdominal skin, while in O a fragment of pancreas was left behind in the peritoneal cavity.

liver contains no glycogen. Similarly in my experiments the tendency has always been to get less glycosuria in the latter experiments of a series, where the dog is lighter in weight and the liver presumably contains less glycogen.

2. The sugar cannot come from the food as morphine-glycosuria is produced in a state of fast.

3. The sugar may come to a small extent from the proteid metabolism of the body—that is from the muscles chiefly: but this cannot be the main source of its production. For in this connection the contrast between the glycosuria of morphine-narcosis and that following excision of the pancreas is very marked. In the latter where the coefficient of consumption of sugar is certainly lowered, and where the power of the liver and muscles to store up sugar as glycogen is largely lost, there is never a high degree of glycosuria soon after the operation, but it takes some hours before it rises to a maximum which is then more or less steadily maintained. Thus Lépine, after excising the pancreas in 52 dogs, observed a maximum glycosuria about the 16th hour, but none at all before the 4th[38].

4. As Claude Bernard showed that diabetic puncture produces its effect on the liver by way of the spinal cord and the great splanchnic nerves, and as we know how morphine proves its presence in the body by an almost immediate action on the nervous centres—shown in vomiting—it is tempting to picture the glycogen of the liver being converted into sugar under nervous influences and to think of the sugar passing into the blood in much the same way as one injects sugar into the circulation in an experiment.

But one thing is certain and that is, that the mechanism is not so simple as this. For, as my experiments in the first part of this paper have shown, injected sugar comes out almost completely in the first hour after the injection. The injected sugar diffuses away throughout the body and raises the sugar contents of the blood, lymph and tissues, dividing itself between all three. The sugar is gradually used up in the body with the aid of the internal secretion of the pancreas, and only as long as the sugar contents of the blood are above the normal is sugar excreted.

It is a very different story in morphine-glycosuria. As practically every one of my experiments shows, the maximum excretion of sugar takes place approximately in the second hour after the injection of morphine: while immediately after the injection, that is in the first half hour or so, very little sugar is excreted.

This is well shown in all three experiments on O: given in Table VII, viz.:

Exp.	First ½ hr.	Next ½ hr.	Next 1½ hrs.	Next 1½ hrs.
O 1	2·55 gm.		3·24 gm.	1·36 gm.
O 2	·005 gm.	2·12 gm.	3·88 gm.	2·41 gm.
	2·125 gm.		(1 hr. only)	
O 3	·86 gm. [½ hr.]		2·09 gm. [½ hr.]	2·46 gm. [1 hr.]

As typical of the glycosuria-effect of morphine-narcosis in a normal dog, I also give here the figures obtained in experiment C 3 (Table IV).

C 3. Experiment after a day's fast. Sept. 25th, 1901. Weight 6·12 kg. Fed last on evening of Sept. 23rd, on meat and biscuits. [In calculating the quantity of excreted sugar, the R.R. of urine before the experiment was taken as ·3 %.]

9.20 a.m.	Bladder emptied.		
9.15 a.m.	Injection of morphine ·007 gm. per kilo.		
10.20 a.m.	Catheter: 6 c.c. urine R.R.	7·3 %	= ·42 gm. in 1 hr.
11.20 a.m.	,, 11 ,, ,,	12·2 %	= 1·31 gm. in 1 hr.
12.20 p.m.	,, 7·6 ,, ,,	10·56 %	= ·78 gm. in 1 hr.
2.20 p.m.	,, 7·5 ,, ,,	7·14 %	= ·51 gm. in 2 hrs.
5.20 p.m.	,, 9·8 ,, ,,	1·05 %	= ·07 gm. in 3 hrs.
		Total	3·09 gm. in 8 hrs.

This fact that the maximum excretion of sugar during morphine-narcosis occurs in the second hour after giving the morphine might appear at first sight to be against the view that the sugar comes from hepatic glycogen. But I think it is partly explained when we consider:

(1) That probably the quantity of sugar thrown by the liver into the circulation is not as great as occurs post-mortem and may be rather small; and that in consequence it takes half an hour or more before the saturation limit is reached. In this connection it is interesting to recall an experiment of Blumenthal, who found that an injection of ·5 gm. of glucose per minute into the ear-vein of a rabbit lasting for 5 minutes (2·5 gm. in all) caused no glycosuria, while an injection of ·3 gm. of glucose per minute lasting for 10 minutes caused very pronounced glycosuria, even distinctly greater than the glycosuria caused by an injection of ·54 gm. of glucose per minute lasting for 5 minutes.

(2) That morphine, ether and anæsthetics in general are protoplasmic poisons and lower the activity of all tissues; and that while morphine produces an immediate effect on the nervous system—vomiting nearly always occurs within five minutes of injecting morphine—its direct depressing effect on the liver and other tissues probably takes some time to reach a maximum. For instance the bowels are

paralysed for hours after morphine, and it is impossible to study satisfactorily the mechanism of peristalsis in a morphinised animal; and similarly it often takes an hour for morphine-narcosis to be fully produced. Consequently if the depressing influence of the morphine does not attain a maximum for some time, the amount of sugar formed in the liver would probably not reach a maximum till the second hour.

I do not propose here to discuss the disputed question of the "glycogenic function" of the liver. What is clear, as both Pavy[19] and his opponents admit, is, that the liver takes sugar out of the blood and stores it up as glycogen; and that this glycogen is converted into sugar post-mortem, and, through nervous influences acting directly or indirectly on the liver, after "diabetic puncture." For the sake of my explanation it is immaterial whether one regards the liver as converting glycogen into sugar under a nervous or chemical stimulus, or whether one regards the liver as so depressed by the influence of morphine—either directly on the liver or indirectly on the nervous system—that the glycogen linked on to the protoplasm of the liver cells can be converted by the diastatic enzymes of the lymph into sugar.

At the same time there are difficulties in looking to the glycogen of the liver as the only source of the sudden production of sugar in morphine-narcosis. For I have not been able to make out any proportion between the degree of glycosuria and the quantity of glycogen found in the liver in the few experiments where I have made an analysis of hepatic glycogen (*see* Table VIII below). Therefore there is much to be said for admitting the interaction of some other factor like the internal secretion of the suprarenal capsules, a subject to which I shall return after describing my experiments on cats.

Other factors in anæsthetic and experimental glycosuria. There is one fact in particular in my experiments which wants explaining, and that is how it was that in A 5 and B 5, each respectively the last of a series of experiments, there was much more sugar excreted after 60 hours' fast than in the previous three experiments. The explanation is of course bound up with the fact that in each of them, unlike the previous experiments of the series, a fistula of the thoracic duct was established[1]: in other words, that the combined influence of ether and

[1] In A 5 the operation was done (1¼ hrs. after morphine) under the influence of a considerable quantity of ether, because in this dog morphine had never a pronounced narcotic effect; while in B 5 only a few whiffs of ether were given, as B was a dog easily narcotised by morphine.

TABLE VIII. *Quantity of sugar excreted by dogs, in which a fistula of the thoracic duct had been established under the influence of chloroform or A.C.E. (or ether), and, in most cases, of morphine also (subcutaneous injection of ·005 gm. to ·01 gm. per kilo).*

Exp.	Weight and state of digestion	Rate lymph flow	Experimental procedure	Quantities of urine and sugar excreted from commencement of giving A.C.E.	Remarks
I	6·5 kilo. Digesting fatty meat	105 c.c. in 5½ hrs.	·06 gm. morph. HCl. Interval 45 mins. Fistula under A.C.E. Chyle flowing in 1 hr.	45·2 c.c. R.P. 1·86 %=·75 gm. in 6½ hrs.	S At end of exp. the R.P. of blood was ·139 %.
II	12 kilo. Fasting	—	·054 gm. morph. HCl. Interval 15 mins. A.C.E. Ether. Fistula. Clear lymph flowing in 45 mins.	61 c.c. R.P. ·55 %=·21 gm. in ½ hr. 31 c.c. R.P. ·68 %=·21 gm. in 6½ hrs. Total ·42 gm. in 7½ hrs.	S At end of exp. the R.P. of blood was ·131 %.
III	Digesting fatty meat	—	·05 gm. morph. HCl. Interval 1½ hrs. A.C.E. Ether. Fistula. Chyle flowing in ½ hr.	13·8 c.c. R.P. ·4 %=·02 gm. in ½ hr. 50 c.c. R.P. ·87 %=·43 gm. in 5½ hrs. Total ·45 gm. in 5½ hrs.	S
IV	Digesting fatty meat	300 c.c. in 5 hrs.	·12 gm. morph. HCl. Interval 1½ hrs. A.C.E. Ether. Fistula. Chyle flowing in 1 hr.	185 c.c. R.P. ·43 %=·37 gm. in 3 hrs. 20 c.c. R.P. ·61 %=·12 gm. in 3 hrs. Total ·49 gm. in 6 hrs.	S
V	Digesting fatty meat	—	No morphine. $CHCl_3$. A.C.E. Fistula. Chyle flowing in 1 hr.	9·5 c.c. R.P. ·31 %=·09 gm. in 1 hr. 51 c.c. R.P. ·3 %=·15 gm. in 3½ hrs. Total ·24 gm. in 4½ hrs.	S
VI	14·9 kilo. Fasting	—	·074 gm. morph. HCl. Interval 2½ hrs. A.C.E. Fistula. Lymph flowing in ½ hr.	18·6 c.c. R.P. 10·52 %=1·92 gm. in 3½ hrs.	S
VII	23·75 kilo. In good condition. Last meal dog-biscuits 24 hrs. before	120 c.c. in 3½ hrs.	·117 gm. morph. HCl. Interval 1½ hrs. A.C.E. Ether. Fistula. Lymph (cloudy) was flowing in 30 mins.	560 c.c. R.P. ·73 %=2·8 gm. in 1 hr. 15·5 c.c. R.P. ·62 %=·09 gm. in 1 hr. 6·9 c.c. R.P. ·65 %=·04 gm. in ¾ hr. Total 2·93 gm. in 2¾ hrs.	C
VIII	Digesting fatty meat	—	$CHCl_3$. A.C.E. followed by ·06 gm. morph. HCl. Fistula. Chyle in 45 mins.	Micturated in going under 40 c.c. R.P. <·5 %=·16 gm. in 6 hrs.	S

IX	Fasting	120 c.c. in 6½ hrs.	$CHCl_3$. A.C.E. followed by ·08 gm. morph. HCl. Fistula. Lymph flowing in 50 mins.	16·5 c.c. R.P. ·75 % = ·08 gm. in 1 hr. 47·5 c.c. R.P. 6·02 % = 2·85 gm. in 4½ hrs.† 4·8 c.c. R.P. 2·5 % = ·12 gm. in 2½ hrs. Total 3·05 gm. in 7½ hrs. † This urine yielded glucosazone crystals.	C
X	14·2 kilo. Fasting	225 c.c. in 7 hrs.	$CHCl_3$ followed by ·08 gm. morph. HCl and A.C.E. Perineum slit. Fistula. Lymph flowing in 1 hr. 150 c.c. blood taken from carotid 100 c.c. of serum from clotted thoracic lymph injected into femoral vein	12 c.c. R.P. ·72 % = ·06 gm. in ¼ hr. 4 c.c. R.P. ·96 % = ·03 gm. in 1 hr. 10·5 c.c. R.P. 7·93 % = ·83 gm. in 1 hr.* 5·9 c.c. R.P. 5·65 % = ·33 gm. in 1 hr. 11·6 c.c. R.P. 2·3 % = ·26 gm. in 1½ hrs. 14·3 c.c. R.P. ·77 % = ·11 gm. in 1½ hrs. 8 c.c. R.P. ·06 % = ·06 gm. in 1½ hrs. Total 1·68 gm. in 7½ hrs.	C R.P. of blood ·26 %. At end of exp. the R.P. of blood was ·16 %. The liver contained 4·15 ‰ glucose, 17·06 ‰ glycogen.
XI	21 kilo, very thin. Fasting	130 c.c. in 2½ hrs.	$CHCl_3$ followed by A.C.E. and ·117 gm. morph. HCl. (F) 200 c.c. blood taken from carotid 50 c.c. warm normal saline injected into femoral vein Fistula in th. duct. Lymph flowing 8½ hrs. after commencement ... ... 50 c.c. normal saline injected into femoral vein ... ... 200 c.c. blood from carotid. 100 c.c. saline injected ...	13·2 c.c. R.P. ·47 % = ·02 gm. in ½ hr. 11 c.c. R.P. ·22 % = < ·02 gm. in 2½ hrs. 43 c.c. R.P. ·24 % = < ·10 gm. in ½ hr. 18 c.c. R.P. ·16 % = < ·02 gm. in 1½ hrs. 36 c.c. R.P. ·19 % = < ·07 gm. in 2½ hrs. Total. Less than ·23 gm. in 7½ hrs.	F
XII	16·2 kilo, young dog, about 10 months old. Fasting	140 c.c. in 4 hrs.	$CHCl_3$ followed by A.C.E. and ·081 gm. morph. HCl. (F) Fistula. Lymph flowing in 1½ hrs.	74 c.c. R.P. ·62 % = ·29 gm. in 1 hr. 71 c.c. R.P. < ·25 % = < ·3 gm. in ½ hr. 29 c.c. R.P. < ·5 % = < ·3 gm. in 1½ hrs. 29·5 c.c. R.P. < ·4 % = < ·3 gm. in 1 hr. 23 c.c. R.P. < ·35 % = < ·3 gm. in 2½ hrs. Total. Less than ·59 gm. in 6½ hrs.	F

TABLE VIII. (*Continued.*)

Exp.	Weight and state of digestion	Rate lymph flow	Experimental procedure	Quantities of urine and sugar excreted from commencement of giving A.C.E.	Remarks
XIII	13·5 kilo, thin and old. Very late stage of digestion	100 c.c. in 2 hrs.	$CHCl_3$ followed by A.C.E. and ·081 gm. morph. HCl. Fistula. Lymph flowing in 1 hr., was cloudy at first, clear afterwards	56 c.c. R.P. < ·5 % = about ·05 gm. in 1½ hrs. 10 c.c. R.P. < ·62 % = about ·04 gm. in 1 hr. 28 c.c. R.P. < ·62 % = about ·17 gm. in 3½ hrs. Total (about) ·26 gm. in 5½ hrs.	C
XIV	Good condition. Fasting	80 c.c. in 4 hrs.	$CHCl_3$ followed by A.C.E. and ·08 gm. morph. HCl. (F) Fistula. Lymph flowing in 1½ hrs.	10 c.c. R.P. < ·3 % = 0 gm. in ½ hr. 32 c.c. R.P. 1·7 % = ·54 gm. in 1 hr. 8·3 c.c. R.P. 1·4 % = ·11 gm. in ½ hr. 34 c.c. R.P. < ·3 % 28 c.c. R.P. < ·3 % } = ·05 gm. in 1 hr.; gm. in 2 hrs. Total ·7 gm. in 5½ hrs.	F Liver contained 8·13 ‰ of glycogen.
XV	19·5 kilo, thin and old. Fasting	80 c.c. in 2 hrs.	$CHCl_3$ followed by A.C.E. and ·06 gm. morph. HCl. (F) Fistula. Lymph flow began in 70 mins.	19 c.c. R.P. < ·5 % = ·01 gm. in ½ hr. 9 c.c. R.P. ·8 % = ·07 gm. in ½ hr. 13·2 c.c. R.P. 1·37 % = ·18 gm. in 1 hr. 7 c.c. R.P. 1·16 % = ·08 gm. in 1 hr. 5 c.c. R.P. 1·04 % = ·05 gm. in ½ hr. Total ·39 gm. in 4 hrs.	F
XVI	12·5 kilo. Digesting fatty meat and biscuits	80 c.c. in 2½ hrs.	$CHCl_3$ followed by A.C.E. and ·04 gm. morph. HCl. (F) Fistula. Chyle flowing in 1 hr.	8 c.c. R.P. < ·41 % = 0 gm. in ½ hr. 7·7 c.c. R.P. ·77 % = ·06 gm. in 1½ hrs. 7 c.c. R.P. ·96 % = ·06 gm. in 1 hr. Total ·12 gm. in 3½ hrs.	F
XVII	19·2 kilo, good condition. Fasting	140 c.c. in 3 hrs.	$CHCl_3$ followed by A.C.E. and ·08 gm. morph. HCl. Fistula. Lymph. flowing in 1 hr.	52 c.c. R.P. ·45 % = ·10 gm. in ½ hr. 9·6 c.c. R.P. ·69 % = ·06 gm. in ½ hr. 10·5 c.c. R.P. ·28 % = ·03 gm. in 1 hr. 24 c.c. R.P. ·4 % = ·09 gm. in 1 hr. Total ·28 gm. in 3½ hrs.	C Liver contained 19·89 ‰ of glycogen.
XVIII	20·3 kilo. Fasting	350 c.c. in 3½ hrs.	$CHCl_3$ followed by A.C.E. and ·14 gm. morph. HCl. (F) Fistula in th. duct. Lymph flowing in 1½ hrs.	Bladder apparently empty after 1 hr. 15 c.c. R.P. 1·22 % = ·18 gm. in ½ hr. 17 c.c. R.P. 2·63 % = ·44 gm. in ½ hr.* 21 c.c. R.P. 3·33 % = ·70 gm. in 1 hr.* 17 c.c. R.P. 2·45 % = ·41 gm. in 1 hr.* 12·5 c.c. R.P. ·8 % = ·10 gm. in ½ hr. Total 1·83 gm. in 5½ hrs.	F At end of exp. the R.P. of blood was ·11 %. The liver contained 3·18 ‰ of glycogen and markedly less than 3 ‰ of glucose.

XIX	16·5 kilo, fat and strong. Fasting	84 c.c. in 2¾ hrs.	No morphine. $CHCl_3$ and A.C.E. Fistula. Lymph flow began in 1 hr.	19 c.c. R.P. < ·18 % = 0 gm. in ½ hr. 18 c.c. R.P. < ·26 % = 0 gm. in 1 hr. 12 c.c. R.P. < ·46 % = ab. ·04 gm. in 1 hr. 6·5 c.c. R.P. < ·48 % = ab. ·04 gm. in 1 hr. 2·4 c.c. R.P. < ·41 % = ab. ·04 gm. in ½ hr. Total (about) ·04 gm. in 3½ hrs.	C Liver contained 10·5 ‰ of glycogen.
XX	22·75 kilo. Fasting	131 c.c. in 3 hrs.	·16 gm. morph. HCl. Interval 2 hrs. $CHCl_3$ and A.C.E. Fistula. Lymph flowing in 1 hr.	42 c.c. R.P. ·61 % = ·16 gm. in ½ hr. 16·2 c.c. R.P. 5·5 % = ·88 gm. in ½ hr.* 12·7 c.c. R.P. 7·8 % = ·99 gm. in 1½ hrs.* 13·6 c.c. R.P. 3·7 % = ·5 gm. in 1 hr.* 13·2 c.c. R.P. 1·2 % = ·15 gm. in 1½ hrs.* Total 2·68 gm. in 4½ hrs.	C The liver contained 26·37 ‰ of glycogen.
XXI	13·5 kilo. Fasting	72·5 c.c. in 4 hrs.	·2 gm. morph. HCl. Interval 2 hrs. $CHCl_3$ and A.C.E. Perineum split. Fistula. Lymph flowing in 1 hr. About 35 c.c. blood from fem. art. taken ... ... Further injection of ·1 gm. morph. HCl ... ... Collection of saliva from subMAX. gland by stimulating C.T.	25 c.c. R.P. 2·63 % = ·60 gm. in ½ hr.* 48 c.c. R.P. 3·84 % = 1·65 gm. in ½ hr.* 9·2 c.c. R.P. 4·16 % = ·38 gm. in 1 hr.* 19·1 c.c. R.P. 3·7 % = ·70 gm. in 1 hr.* 11·7 c.c. R.P. 1·72 % = ·20 gm. in 1 hr. 6·2 c.c. R.P. ·69 % = ·04 gm. in 1 hr. 12·5 c.c. R.P. 1·39 % = ·17 gm. in 1½ hrs. Total 3·74 gm. in 6½ hrs.	C R.P. of blood was ·126 %.
XXII	20·75 kilo, good condition. Fasting	145 c.c. in 3½ hrs.	·13 gm. morph. HCl. Interval 1½ hrs. $CHCl_3$ and A.C.E. (F) Fistula. Lymph flowing in 1 hr. About 40 c.c. blood taken from fem. art. for estimation ...	172 c.c. R.P. 1·13 % = 1·54 gm. in ½ hr. 33 c.c. R.P. 4·8 % = 1·58 gm. in ½ hr.† 17·3 c.c. R.P. 5·77 % = ·99 gm. in ½ hr. 10·9 c.c. R.P. 1·06 % = ·11 gm. in 1½ hrs. 7·8 c.c. R.P. ·35 % = ·02 gm. in 1 hr. Total 4·24 gm. in 4½ hrs. † The R.P. was 3·67 % on estimation with polarimeter.	F The R.P. of blood was considerably more than ·13 %. The liver weighed 496 gm. and contained 16·8 ‰ of glycogen.

TABLE VIII. (*Continued.*)

Exp.	Weight and state of digestion	Rate lymph flow	Experimental procedure	Quantities of urine and sugar excreted from commencement of giving A.C.E.	Remarks
XXIII	15·5 kilo. Digesting fatty meat	—	·2 gm. morph. HCl. Interval 30 mins. Ether. Fistula. Lymph flowing in 1½ hrs.	Urine had R.P. ·33 % in half an hr. 38 c.c. R.P. 1·53 % = ·58 gm. in 4 hrs.	S
P	11·15 kilo. Perineum split one week before under anæsthetic. Fed evening before with fatty meat	—	·078 gm. morph. tart. Interval 2 hrs. A.C.E. and ether. Fistula. Lymph flowing in 1½ hrs. No more anæsthetic	Dog micturated. Urine lost 5 c.c. R.P. 1·5 % = ·07 gm. in 1½ hrs. 124 c.c. R.P. ·57 % = ·45 gm. in 21½ hrs. 400 c.c. R.P. ·22 % = 0 gm. in 23½ hrs. 90 c.c. R.P. ·16 % = 0 gm. in 1½ hrs. Total ·52 gm. in 28 hrs. and none subsequently	C In these two exps. the operation of establishing a fistula of the th. duct was done with antiseptic precautions, the dogs being allowed to recover from the anæsthetic and not killed for two days.
I	10·75 kilo. Perineum split some months before. Fed 24 hrs. previously on meat	—	No morphine. $CHCl_3$ followed by A.C.E. and ether. Fistula. Lymph flowing in 1½ hrs. No more anæsthetic	Bladder emptied in going under 30 c.c. R.P. 1·6 % = ·48 gm. in 2 hrs. 254 c.c. R.P. ·35 % = ·38 gm. in 26½ hrs. 263 c.c. R.P. ·2 % = 0 gm. in 19½ hrs. Total ·86 gm. on 1st day and none subsequently	The R.P. of normal urine was taken as ·2 % throughout.

In calculating the quantity of sugar excreted, ·2 % is deducted from the R.P. of the first urine obtained to allow for any urine which may have been in the bladder before the experiment commenced. No allowance is made in the case of the other urines.

An asterisk marks those urines tested with yeast, which led to fermentation.

Abbreviations:

In the above table F implies that the urine was obtained by a catheter inserted into a fistula of the urethra close to the bladder, the animal being a male.

" " C implies the use of a catheter in a bitch, usually with a split perineum.

" " S implies that the urine was squeezed from the bladder by 2 or 3 fingers inserted through a small hole in the linea alba, the animal being a male.

nervous disturbance, produced by the operation of establishing a fistula of the thoracic duct, had an added effect on the sugar-producing mechanism. For Biedl[20] in 1898 published some experiments showing that fistula or ligature of the thoracic duct often produced a transitory glycosuria, lasting some hours or days. He, however, failed to produce it in 33 % of cases of ligature and 11 % of cases of fistula, and though he thought he had shown the glycosuria was not due to the anæsthetic but was genuinely due to the fact that chyle was prevented from entering the circulation, subsequent workers have failed to substantiate his view. I myself in the course of numerous experiments made chiefly in 1899—1900, in which I injected the thoracic lymph of digesting and fasting dogs into cats, was able to study the glycosuria associated with fistula of the thoracic duct in dogs, and I found no difference either in the degree or in the course of the excretion between the glycosuria associated with fistula of the thoracic duct and that caused by morphine. Thus the maximum excretion of sugar took place in both cases during the second and third hours after the beginning of the experiment (as in Exp. X, Table VIII) and not later, while the greatest quantity of sugar excreted during a whole experiment amounted in one case to 4·24 gm. (Table VIII, Exp. XXII), in the other case to 4·35 gm. (Table VI, Exp. K 1). Lastly in some cases of fistula of the thoracic duct (for example Experiments VI, X, and XX) I obtained urines containing a high percentage of sugar though Biedl gives 5·8 % as the highest percentage he obtained; but then perhaps he did not use morphine.

My experiments are recorded in Table VIII and they seem to prove much the same points as I have already been discussing in relation to ether and morphine-glycosuria, viz., individual variation and the difficulty of attributing any influence on the glycosuria to a state of digestion or fasting. If anything, glycosuria seems to have occurred more readily in a fasting than in a digesting animal, but there was no uniformity about the experiments suggesting any certain connection between degree of glycosuria and the fasting state. Rather do these experiments illustrate very well the point I have already made in reference to Experiments A 5 and B 5 that the operative procedure of establishing a fistula of the thoracic duct sets up such a stimulation of nerves that a glycosuria may occur in spite of fasting and a lowered body-weight.

On the whole then there is little evidence that Biedl's explanation of the glycosuria he described is right, though if the internal secretion

of the pancreas finds its way into the circulation by way of the thoracic duct, it is theoretically probable that fistula of the duct would have some influence in the direction of producing glycosuria. How easy it is to be deceived by single experiments done on individual animals I proved for myself when I began this research on glycosuria ten years ago and published a preliminary note suggesting that glycosuria could be produced in cats by injecting thoracic lymph from a dog[m]. This result, based on about forty experiments, was completely erroneous as subsequent experiments proved; the glycosuria there described was due in all cases to experimental conditions (anæsthetic and stimulation of nerves) and not to the injection of lymph and is probably identical with the "Binding-down Glycosuria" (Fesselungsglycosurie der Katzen) described by Böhm and Hoffmann[m]. These experiments were on cats: and, although I did some ten control experiments, it just happened that in these ten I obtained little or no glycosuria for various reasons; but chiefly because they happened to be individuals which I had only just obtained and had not fed up in the laboratory before experimenting, so that they were thin and in poor condition.

I have no doubt in fact that the mechanism of experimental glycosuria in cats is very similar to that of morphine-narcosis in dogs; as will be seen from the experiments given in Table IX.

In suggesting that the operative procedure of establishing a fistula of the thoracic duct gives rise to a nervous stimulus resulting in the production of glycosuria, I do not pretend that I have proved what nerves are stimulated or what paths the nervous impulses take, leading to the production of glycosuria; but besides the left vagus and recurrent laryngeal nerves the sympathetic nerve in particular is in the neighbourhood of the junction of the left subclavian and jugular veins, and it or its branches may therefore be disturbed by the operative procedure even if other nerves are unaffected. Similarly in cats disturbance of nerves as well as anæsthetisation seems to have a definite influence in producing glycosuria. For in some of my later experiments I deliberately ligatured the splenic artery and attendant nerves or put a pair of Spencer-Wells forceps on the pancreas in order to set up stimulation of nerves (the pancreas in the cat is covered with Pacinian corpuscles) or to produce "shock" in the pancreas; and on the whole I obtained in these experiments a greater degree of glycosuria. For, if we compare the six experiments, 1, 8, 9, 10, 11 and 12 (Table IX) with the nine experiments, 2, 3, 4, 5, 7, 13, 14, 15 and 16 which lasted from 1 hr. 25 mins. to 2 hrs. 10 mins. and were all on cats fed up in the laboratory

in good condition and in a state of digestion, we find that the first lot give an average excretion during the experiment of ·35 gm. (in 1 hr. 50 mins.) while the second lot give an average excretion of ·17 gm. (in 1 hr. 50 mins.). But these are small figures compared to those obtained in morphine-glycosuria in dogs; and the difference is not great enough to draw any certain conclusion without comparing a greater number of animals experimented on for identical lengths of time.

These experiments suggest to me the need of saying a little more about glycosuria in cats. I did a great number of experiments to see if there was any difference in the effect of injecting chyle and the thoracic-duct lymph from a fasting dog respectively, and between injecting blood-serum from digesting and fasting animals respectively; and I came to the conclusion ultimately that what I injected whether it was thoracic lymph-serum, blood-serum or normal saline had very little effect on glycosuria as will be seen from the experiments recorded in Tables IX, X, XI and XII.

In most cases the cats had been fed up previously on a diet of meat and milk alternating every other day with bread, milk and sugar (about 20 gm.): and some of which had been given sugar dissolved in milk on the morning of the experiment or may have drunk some sugary milk left over from the evening before (as Exp. 1, Table X).

In the course of these experiments I made a large number of analyses of the glycogen of the liver and in Table XII I have arranged the order of the experiments so as to see if any connection can be made out between the degree of glycosuria and the quantity of carbohydrate in the liver.

My estimation of glycogen was made according to the method employed by Pavy[29]; that is, I rapidly excised the liver, minced a portion in a sausage machine, added from 20 gm. to 30 gm. of the pap to a weighed quantity of 96 % alcohol, carefully weighed again and then stirred up with another 200 c.c. of alcohol. After filtering off the alcoholic extract (containing the sugar) and extracting with at least two more lots of boiling alcohol, I heated the residue with a small quantity of 10 % potash, using a reflux condenser till all was dissolved. I then precipitated the glycogen with an excess of alcohol and, after carefully washing the precipitate with saline alcohol, dissolved it in 2·2 % HCl. The solution after inversion with a reflux condenser was then made up to known bulk and estimated with Pavy's fluid. In Table XII I have arbitrarily, for the purposes of a rough comparison, assumed the amount of sugar present at the moment of excising the liver from a cat, that is

TABLE IX. *The quantity of sugar excreted in cats in experimental glycosuria (injection of 12 c.c. to 20 c.c. normal saline into the splenic vein, while the anæsthetic employed was A.C.E. followed by ether given through a trachea-tube).*

Number of exp.	Weight of cat. Time between last food and beginning of exp.	Experimental procedure	Quantities of urine and sugar excreted during experiment	Carbohydrate in blood and liver at end of exp.
1	2·5 kilo. 4½ hrs.	Cannula in splenic vein. Ligature of splenic artery with attendant nerves. Injection of warm normal saline at intervals during an hour [20 c.c. in all]. Last injection 35 mins. before taking blood	·5 c.c. not estimated in 15 mins. 1·3 c.c. R.P. 3·66 % = ·047 gm. in 15 mins. 6·1 c.c. R.P. < ·69 % = ·08 gm. in 90 mins. C Total ·07 gm. in 2 hrs.	R.P. of blood ·206 % (·207 %). Liver 21·84 ‰ of glycogen.
2	3·1 kilo. 4½ hrs.	Cannula in s. v. [nerves with artery not ligatured]. Injections during 1 hr. [14 c.c. in all] ending 40 mins. before taking blood	Bladder apparently empty in 30 mins. 1·2 c.c. R.P. 4·33 % = ·051 gm. in 45 mins. 4·4 c.c. R.P. 4·76 % = ·209 gm. in 60 mins. S Total ·26 gm. in 2½ hrs.	R.P. of blood ·22 %. Liver 13·54 ‰ of glycogen.
3	2·6 kilo. 6½ hrs.	Nerves and art. dissected away from the vein but not ligatured. Injections during 50 mins. [12 c.c. in all] ending 50 mins. before taking blood	13 c.c. R.P. ·26 % = ·007 gm. in 15 mins. 2·4 c.c. R.P. ·22 % = ·005 gm. in 15 mins. 2·9 c.c. R.P. ·20 % = ·005 gm. in 30 mins. 2·7 c.c. R.P. ·3 % = ·008 gm. in 70 mins. C Total ·02 gm. in 2 hrs. 10 mins.	R.P. of blood ·16 % (·158 %). Liver 15·8 ‰ of glycogen.
4	2·4 kilo. Fed evening before	No disturbance of art. and nerves. Injections during 30 mins. [12 c.c. in all] ending 1 hr. before taking blood	23 c.c. R.P. < ·2 % = 0 gm. in 15 mins. 1·9 c.c. R.P. 3·38 % = ·064 gm. in 15 mins.* 4·5 c.c. R.P.† 6·61 % = ·297 gm. in 30 mins.* 3·1 c.c. R.P. 4·27 % = ·132 gm. in 50 mins.* C Total ·49 gm. in 1 hr. 50 mins. † R.P. of this urine with polarimeter was 5·84 %.	R.P. of blood ·22 % (·218 %). Liver 23·13 ‰ of glycogen.
5	2 kilo. 7¾ hrs.	As previous	24·8 c.c. R.P. ·89 % = ·173 gm. in 20 mins.* 7·1 c.c. R.P. ·82 % = ·058 gm. in 35 mins. 8 c.c. R.P. 1·07 % = ·085 gm. in 60 mins.* S Total ·31 gm. in 1 hr. 55 mins.	Liver 8·3 ‰ of glycogen.

6	2 kilo. Thin and in poor condition 6½ hrs.	As previous. Hæmorrhage from spleen during exp.	4·8 c.c. R.P. ·54 % = ·016 gm. in 20 mins. 2·1 c.c. R.P. 2·3 % = ·048 gm. in 105 mins. C Total ·06 gm. in 2 hrs. 5 mins.	Liver 1·33 ‰ of glycogen.
7	2·15 kilo. 6½ hrs.	As previous. Ending 40 mins. before taking blood	21 c.c. R.P. ·32 % = ·025 gm. in 25 mins. 5·5 c.c. R.P. ·31 % = ·017 gm. in 50 mins. 2·3 c.c. R.P. ·39 % = ·008 gm. in 50 mins. S Total ·05 gm. in 2 hrs. 5 mins.	R.P. of blood ·23 % (·24 %). Liver 2·96 ‰ of glycogen.
8	2·75 kilo. 6½ hrs.	Forceps on pancreas. Injections during 1 hr. [12 c.c. in all] ending 35 mins. before taking blood	4·1 c.c. R.P. ·48 % = ·011 gm. in 10 mins. 1·9 c.c. R.P. 4·4 % = ·063 gm. in 15 mins. 2·6 c.c. R.P. 5·06 % = ·131 gm. in 45 mins. 2·6 c.c. R.P. 2·95 % = ·076 gm. in 45 mins. S Total ·3 gm. in 1 hr. 55 mins.	R.P. of blood ·174 % (·168 %). Liver 30·76 ‰ of glycogen.
9	2·5 kilo. 6 hrs.	As previous. Ending 28 mins. before taking blood	43 c.c. R.P. ·29 % = ·038 gm. in 15 mins. 5·9 c.c. R.P. 5·12 % = ·302 gm. in 25 mins. 3·5 c.c. R.P. 5·82 % = ·203 gm. in 40 mins. 2·2 c.c. R.P. 1·35 % = ·029 gm. in 50 mins. S Total ·57 gm. in 2 hrs. 10 mins.	R.P. of blood ·158 % (·167 %). Liver 13·67 ‰ of glycogen.
10	3 kilo. 6½ hrs.	As previous. Ending 25 mins. before taking blood	16 c.c. R.P. ·2 % = 0 gm. in 10 mins. 3·2 c.c. R.P. 5·05 % = ·161 gm. in 17 mins. 6·5 c.c. R.P. 8·54 % = ·555 gm. in 68 mins. S Total ·71 gm. in 1 hr. 35 mins.	R.P. of blood ·214 % Liver 19·18 ‰ of glycogen.
11	2·15 kilo. 6½ hrs.	As previous. Ending 20 mins. before taking blood	9·3 c.c. R.P. ·53 % = ·03 gm. in 15 mins. 1 c.c. R.P. 4·9 % = ·049 gm. in 15 mins. 2 c.c. R.P. 1·31 % = ·026 gm. in 70 mins. S Total ·1 gm. in 1 hr. 40 mins.	R.P. of blood ·184 % (·195 %). Liver 22·26 ‰ of glycogen.
12	2·15 kilo. 6½ hrs.	As previous. Ending 35 mins. before taking blood	Bladder emptied before experiment 2·15 c.c. R.P. 3·5 % = ·075 gm. in 37 mins. 4·9 c.c. R.P. 6·2 % = ·303 gm. in 78 mins. S Total ·37 gm. in 1 hr. 55 mins.	R.P. of blood ·211 % (·203 %). Liver at least 22·47 ‰ of glycogen.

TABLE IX. (*Continued.*)

Number of exp.	Weight of cat. Time between last food and beginning of exp.	Experimental procedure	Quantities of urine and sugar excreted during experiment	Carbohydrate in blood and liver at end of exp.
13	3·2 kilo. 6½ hrs.	No forceps put on pancreas. Injections during 40 mins. [14 c.c. in all] ending 23 mins. before taking blood	61 c.c. R.P. ·28 % = ·04 gm. in 15 mins. 1·8 c.c. R.P. ·31 % = ·005 gm. in 10 mins. 2·7 c.e. R.P. ·34 % = ·009 gm. in 60 mins. S Total ·05 gm. in 1 hr. 25 mins.	R.P. of blood ·131 % (·135 %). Liver 11·07 ‰ of glycogen.
14	3·25 kilo 7 hrs.	As previous. Ending 20 mins. before taking blood	33 c.c. R.P. < ·19 % = 0 gm. in 15 mins. 3·3 c.c. R.P. < ·26 % = ·006 gm. in 10 mins. 6·5 c.c. R.P. 1·88 % = ·122 gm. in 65 mins. S Total ·12 gm. in 1 hr. 30 mins.	R.P. of blood ·17 % (·17%). Liver 12·06‰ of glycogen.
15	3·25 kilo. 7 hrs.	As previous. Ending 25 mins. before taking blood	32 c.c. R.P. ·18 % = 0 gm. in 15 mins. 1·3 c.c. R.P. ·63 % = ·008 gm. in 15 mins. 4·3 c.c. R.P. 1·24 % = ·053 gm. in 60 mins. S Total ·06 gm. in 1 hr. 30 mins.	R.P. of blood ·15 %. Liver (about) 12 ‰ of glycogen.
16	4·6 kilo. 6½ hrs.	As previous. Ending 45 mins. before taking blood	23 c.c. R.P. ·24 % = ·009 gm. in 15 mins. 3·1 c.c. R.P. ·93 % = ·038 gm. in 15 mins. 8 c.c. R.P. 1·58 % = ·146 gm. in 90 mins. S Total ·19 gm. in 2 hrs.	R.P. of blood ·174 % (·167 %).

All the cats in the above experiments had been fed up in the laboratory for at least three weeks on bread, milk, sugar and lights, but the last food given consisted only of milk and lights.

In calculating the quantity of sugar excreted ·2 % is deducted from the R.P. of the first urine, if it may have been partly secreted before the experiment commenced. Otherwise no allowance is made for the R.P. of normal urine.

Abbreviations:—The letter S implies that the urine was squeezed from the bladder, while C implies the use of a catheter.

A star placed against any urine implies that it was tested with yeast and showed the presence of sugar by fermenting.

The figures in brackets in the last column express the R.P. of the blood after inversion.

TABLE X. *Effect of injection into cats of lymph-serum from the thoracic duct of dogs. The first* 11 *are all experiments with chyle. (Anæsthetic employed was A.C.E. followed by ether given with a trachea-tube.)*

Number of exp.	Weight of cat. Last food	Experimental procedure	Quantities of urine and sugar excreted	Remarks
1	2·35 kilo. Evening before	Cannula in f. v. 50 c.c. injected during first 2 hrs. of exp.	50 c.c. R.P. ·5 % = ·15 gm. in ½ hr. 20 c.c. R.P. 3·29 % = ·653 gm. in 2½ hrs. 35 c.c. R.P. 2·9 % = 1·015 gm. in 5½ hrs. S Total 1·82 gm. in 8½ hrs.	The exp. began at 8.30 a.m., so cat may have drunk some of sugary milk shortly before exp.
2	Good condition. Evening before	Cannula in s. v. as previous ...	10 c.c. R.P. ·66 % = ·040 gm. in ½ hr. 31 c.c. R.P. ·98 % = ·303 gm. in 4½ hrs. S Total ·34 gm. in 5 hrs.	This exp. did not begin till noon.
3	2·2 kilo. Evening before	Cannula in s. v. 60 c.c. of clotted dog's chyle [kept on ice a week] injected during first hour	9 c.c. R.P. 1·19 % = ·09 gm. in ½ hr. 41·5 c.c. R.P. ·53 % = ·22 gm. in 4 hrs. S Total ·31 gm. in 4½ hrs.	—
4	2·26 kilo. Milk without sugar given 2½ hrs. before exp.	Cannula in f. v. 58 c.c. injected during first 4 hrs. of exp.	20 c.c. R.P. ·11 % = 0 gm. in ½ hr. 29·2 c.c. R.P. 3·42 % = ·99 gm. in 4½ hrs. S Total ·99 gm. in 5 hrs.	—
5	3·5 kilo. Milk without sugar given 5 hrs. before exp.	Cannula in f. v. 52 c.c. injected during first 3 hrs. of exp.	18·5 c.c. R.P. ·35 % = ·027 gm. in ½ hr. 18·5 c.c. R.P. 2·81 % = ·519 gm. in 4 hrs. S Total ·54 gm. in 4½ hrs.	—
6	Milk without sugar given 6½ hrs. before	Cannula in s. v. S. m. v. ligatured. 20 c.c. of chyle and 20 c.c. of normal saline injected during first hour	60 c.c. R.P. ·2 % = 0 gm. in ½ hr. 5·9 c.c. R.P. 4·3 % = ·253 gm. in 1½ hrs. S Total ·25 gm. in 1½ hrs.	—
7	Small and in poor condition. Milk 7 hrs. before exp.	Cannula in s. m. v. 38 c.c. of chyle and 20 c.c. of normal saline injected during first hour	18 c.c. R.P. ·35 % = ·027 gm. in ½ hr. 1·6 c.c. R.P. ·74 % = ·011 gm. in 1½ hrs. S Total ·03 gm. in 2 hrs.	—

TABLE X. (*Continued.*)

Number of exp.	Weight of cat. Last food	Experimental procedure	Quantities of urine and sugar excreted	Remarks
8	Good condition. Milk without sugar given 5½ hrs. before exp.	Cannula in s.v. S.m.a. and vein ligatured. 50 c.c. of chyle and 20 c.c. of normal saline injected during first hour	5·8 c.c. R.P. ·43 % = ·011 gm. in ½ hr. 1·7 c.c. R.P. ·65 % = ·011 gm. in 1½ hrs.	—
			S Total ·02 gm. in 2 hrs.	
9	Fed 7½ hrs. before exp.	Cannula in s.v. 33 c.c. injected during first hour	23 c.c. R.P. ·31 % = ·023 gm. in ½ hr. 4·7 c.c. R.P. 2·31 % = ·108 gm. in 1½ hrs.	—
			C Total ·18 gm. in 1 hr. 50 mins.	
10	Good condition and size. Milk without sugar given 3½ hrs. before exp.	Cannula in s.v. 56 c.c. chyle and 15 c.c. normal saline injected during first 2 hrs. of exp.	11·2 c.c. R.P. 2·5 % = ·257 gm. in ½ hr. 2·4 c.c. R.P. 5·6 % = ·134 gm. in ½ hr. 4·1 c.c. R.P. 5·6 % = ·229 gm. in ½ hr. 2·3 c.c. R.P. 3·3 % = ·075 gm. in ½ hr. 12 c.c. R.P. 3·18 % = ·381 gm. in 1 hr.	—
			S Total 1·07 gm. in 3 hrs.	
11	Good condition and size. Milk without sugar given 2½ hrs. before exp.	Cannula in s.v. 71 c.c. injected during first 3 hrs. of exp.	104 c.c. R.P. ·25 % = ·104 gm. in ½ hr. 3·2 c.c. R.P. 8·65 % = ·276 gm. in 1½ hrs. 3·4 c.c. R.P. 5·07 % = ·172 gm. in ½ hr. 10·4 c.c. R.P. 1·17 % = ·121 gm. in 1½ hrs.	Liver contained 14·31 ‰ of glycogen.
			C Total ·67 gm. in 3½ hrs.	
12	2·3 kilo. Milk 1 hr. before beginning of exp.	Cannula in s.v. Injection of 102 c.c. of lymph from fasting dog during first 3 hrs. of exp.	1·2 c.c. R.P. ·48 % = ·008 gm. in 20 mins. 2·3 c.c. R.P. ·64 % = ·014 gm. in 55 mins. 4·5 c.c. R.P. ·37 % = ·016 gm. in 60 mins. 1·2 c.c. R.P. ·29 % = ·008 gm. in 105 mins.	—
			S Total ·08 gm. in 4 hrs.	
13	2·25 kilo. Thin. Milk 6½ hrs. before exp.	Cannula in s.v. Injection of 50 c.c. of lymph-serum during first 1½ hrs.	13 c.c. R.P. < ·4 % = 0 gm. in 15 mins. 2·8 c.c. R.P. ·83 % = ·023 gm. in 65 mins.	Liver 15·62 ‰ of glycogen.
			C Total ·02 gm. in 1 hr. 20 mins.	

14	2·5 kilo. Milk and lights 8½ hrs. before exp.	Cannula in s. v.; the spl. art. and nerves ligatured. Injection of 37 c.c. lymph-serum. Ligature of s.m.a. with disturbance of nerves. Injection of 25 c.c. lymph-serum	54 c.c. R.P. ·28 %=·04 gm. in 25 mins. 3·9 c.c. R.P. ·36 %=·014 gm. in 25 mins. 1·7 c.c. R.P. ·43 %=·007 gm. in 25 mins. ·6 c.c. R.P. ·8 %=·004 gm. in 75 mins. C Total ·06 gm. in 2 hrs. 30 mins.	Liver 2·47 ‰ of glycogen, and *less than* 3·4 ‰ of sugar.
15	2·75 kilo. Milk and lights and *cane-sugar* 6 hrs. before exp.	Cannula in s. v. 61 c.c. lymph-serum injected during first 2 hrs. of exp. Spl. art. and nerves ligatured	8 c.c. R.P. ·62 %=·032 gm. in 15 mins. ·6 c.c. R.P. ·9 %=·005 gm. in 30 mins. 4·6 c.c. R.P. 5·73 %=·263 gm. in 70 mins. 5·2 c.c. R.P. 4·76 %=·247 gm. in 40 mins. C Total ·54 gm. in 2 hrs. 35 mins.	Liver 17·64 ‰ of glycogen and 5·1 ‰ of sugar.
16	3·25 kilo. Milk and lights 2 hrs. before	Cannula in s. v. Ligature of spl. art. and nerves. Injection of 70 c.c. lymph-serum during first 2 hrs.	35 c.c. R.P. ·2 % = 0 gm. in 30 mins. 2·5 c.c. R.P. ·33 %=·008 gm. in 10 mins. 12 c.c. R.P. 1·37 %=·164 gm. in 60 mins. 9·7 c.c. R.P. 4·34 %=·42 gm. in 65 mins. S Total ·59 gm. in 2 hrs. 45 mins.	Liver 16·69 ‰ of glycogen.
17	2·75 kilo. Fed with milk and lights 2½ hrs. before exp.	Cannula in s. v. Ligature of spl. art. and nerves. Injection of 62 c.c. lymph-serum during first 2 hrs. of exp.	13·7 c.c. R.P. 1·22 %=·137 gm. in 30 mins. 6·1 c.c. R.P. 1·83 %=·111 gm. in 65 mins. 7·7 c.c. R.P. < ·34 %=·007 gm. in 55 mins. 4 c.c. R.P. ·55 %=·022 gm. in 15 mins. S Total ·27 gm. in 2 hrs. 45 mins.	Liver 24·03 ‰ of glycogen.
18	2·6 kilo. Milk and lights 5 hrs. before exp.	Cannula in s. v. Ligature of spl. art. and nerves. Injection of 37 c.c. lymph-serum during first 2 hrs. of exp.	12·6 c.c. R.P. 2·12 %=·252 gm. in 30 mins. 3·2 c.c.† R.P. 11·9 %=·38 gm. in 50 mins. 1·4 c.c. R.P. 13·75 %=·192 gm. in 40 mins. 1·2 c.c. R.P. 3·48 %=·041 gm. in 100 mins. Total ·86 gm. in 3 hrs. 40 mins.	Liver 8·81 ‰ of glycogen.

† An estimation of this urine with the polarimeter also gave an R.P. of 11·9 %.

In calculating the quantity of sugar excreted ·2 % is deducted from the R.P. of the first urine in each experiment. Otherwise no allowance is made for the R.P. of normal urine.

Abbreviations:—The letter S implies that the urine was squeezed from the bladder, while C implies the use of a catheter.

In the first nine experiments I have no record of how long the cats were kept in the laboratory before being used: but the remaining nine were all fed up in the laboratory for periods varying from a week to three months. All the cats were fed on the evening of the day preceding each experiment with lights and milk, which except in the case of Exps. 7 and 9 had 10 gm. of glucose dissolved in it.

TABLE XI. *Experiments on cats, associated with glycosuria.*

(Footnotes to Table IX apply also to this Table. An asterisk marks those experiments in which about 20 gm. of cane-sugar dissolved in milk were given from 4 to 8 hours before the experiment.)

Number of exp.	Weight of cat. Last food	Experimental procedure	Quantity of urine and sugar excreted in experiment	Remarks
1	2·25 kilo. About 8 hrs. before	Cannula in s. v. Injection 50 c.c. whipped and centrifugalised blood-serum from digesting dog in 50 mins.	24·5 c.c. R.P. < ·6 % = ·049 gm. in 55 mins. 3·7 c.c. R.P. < ·5 % = ·011 gm. in 70 mins. 2·7 c.c. R.P. < ·4 % = ·005 gm. in 50 mins. S Total ·06 gm. in 2 hrs. 55 mins.	Liver contained after 2 hrs. incubation with normal saline at 40°C., 12·2 ‰ sugar 4·6 ‰ glycogen.
2	2·5 kilo. About 10 hrs. before	Cannula in s.v. Injection of 87 c.c. of saline extract of lymphatic glands from an ox during 1½ hrs.	26 c.c. R.P. ·4 % = ·052 gm. in 20 mins. 15·5 c.c. R.P. < ·3 % = ·015 gm. in 60 mins. 21 c.c. R.P. < ·3 % = ·02 gm. in 55 mins. S Total ·08 gm. in 2¼ hrs.	The liver was enlarged and markedly cirrhotic. It contained 4·88 ‰ of glycogen.
3	2·5 kilo. Evening before	Cannula in s.v. Injection of 46 c.c. whipped and centrifugalised blood-serum from a digesting cat in 1 hr.	10 c.c. R.P. 1·47 % = ·127 gm. in 20 mins. 28 c.c. R.P. 1·27 % = ·355 gm. in 100 mins. C Total ·48 gm. in 2 hrs.	Liver 15·48 ‰ of glycogen.
*4	3·25 kilo. 5¾ hrs. after meat, milk and cane-sugar	Cannula in s. v. ... ... Injection of 40 c.c. whipped and centrifugalised blood-serum from a digesting dog during 45 mins. A subsequent injection of 30 c.c. serum in 10 mins. almost killed the cat	26 c.c. R.P. ·62 % = ·105 gm. in 15 mins. 9 c.c. R.P. 5 % = ·45 gm. in 25 mins. 14·5 c.c. R.P. 4·45 % = ·645 gm. in 50 mins. S Total 1·2 gm. in 1½ hrs.	Liver 9·69 ‰ of glycogen.
*5	3·75 kilo. 5¾ hrs. as previous	Cannula in s. v. ... ... 50 c.c. of citrated plasma from digesting dog injected during 30 mins. ... ... 30 c.c. of whipped and centrifugalised blood-serum from same dog injected in 30 mins. ... 40 c.c. of blood-serum injected in 15 mins.	29 c.c. R.P. ·7 % = ·145 gm. in 20 mins. 14 c.c. R.P. 5 % = ·7 gm. in 40 mins. 13 c.c. R.P. 3·4 % = ·442 gm. in 50 mins. 4 c.c. R.P. ·9 % = ·036 gm. in 25 mins. S Total 1·32 gm. in 2¼ hrs.	Liver 19·79 ‰ of glycogen.

*6	2·25 kilo. 5½ hrs. as previous	Cannula in s. v. 50 c.c. citrated plasma from fasting cat injected during 1 hr.	21·2 c.c. R.P. ·92 % = ·148 gm. in 15 mins. 23·8 c.c. R.P. 2·22 % = ·528 gm. in 75 mins. 13·9 c.c. R.P. < ·2 % = 0 gm. in 30 mins. S Total ·67 gm. in 2 hrs.	Liver 24·21 ‰ of glycogen.
*7	4 kilo. 8½ hrs. as previous	Cannula in s. v. ... ... Injection of 50 c.c. whipped and centrifugalised blood-serum from digesting dog in 50 mins. ... 50 c.c. serum injected into jugular vein during 15 mins.	26 c.c. R.P. ·46 % = ·067 gm. in 15 mins. 3·7 c.c. R.P. 5·37 % = ·197 gm. in 60 mins. 11 c.c. R.P. 6·58 % = ·723 gm. in 25 mins. 4·8 c.c. R.P. 5·78 % = ·277 gm. in 25 mins. S Total 1·26 gm. in 2 hrs. 5 mins.	There was some loss in estimating the liver, which contained at least 13·5 ‰ of glycogen.
*8	2·9 kilo. 5½ hrs. as previous	Cannula in s. v. Injection of 40 c.c. whipped and centrifugalised blood-serum from digesting dog in 30 mins.	17 c.c. R.P. ·58 % = ·064 gm. in 15 mins. 13 c.c. R.P. 4·16 % = ·540 gm. in 35 mins. 6·2 c.c. R.P. 4·08 % = ·252 gm. in 10 mins. S Total ·85 gm. in 1 hr.	At end of exp. the R.P. of blood was ·27 %. Liver 9·43 ‰ of glycogen.
*9	3·5 kilo. 5½ hrs. as previous	Cannula in s. m. a. with disturbance of nerves ... ... Injection of 30 c.c. normal saline and 20 c.c. blood-serum from digesting dog	27 c.c. R.P. 1·48 % = ·332 gm. in 30 mins. 6·3 c.c. R.P. 6·89 % = ·434 gm. in 60 mins. 7·1 c.c. R.P. 8·19 % = ·581 gm. in 35 mins. S Total 1·34 gm. in 2 hrs. 5 mins.	—
Experiments characterised by disturbance of visceral nerves, in which no injections were given.				
*10	3·25 kilo. 4½ hrs. as previous	Ligature of branch of s. m. a. close to glands at root of mesentery Ligature on art. pulled upon ... Cannula inserted in art. Injection of 25 c.c. dialysed blood-serum of dog	Urine passed in going under had R.P. ·59 % 7 c.c. R.P. 5·2 % = ·364 gm. in 20 mins.* 31 c.c. R.P. 6·58 % = 2·039 gm. in 60 mins.* 13·6 c.c. R.P. 6·58 % = ·894 gm. in 20 mins.* 7·1 c.c. R.P. 7·35 % = ·521 gm. in 20 mins.* 2·2 c.c. R.P. 8·42 % = ·185 gm. in 20 mins.* 6·1 c.c. R.P. 7·69 % = ·469 gm. in 20 mins.* 12·4 c.c. R.P. 7·84 % = ·972 gm. in 50 mins.* S Total 5·44 gm. in 3¼ hrs.	All the samples of urine except first and fifth were passed by cat itself into a beaker.

TABLE XI. (*Continued.*)

Number of exp.	Weight of cat. Last food	Experimental procedure	Quantity of urine and sugar excreted in experiment	Remarks
11	2·75 kilo. 5 hrs. after milk and meat (but no sugar)	It took 10 mins. to empty the bladder by squeezing. A small piece of the ileum which had escaped through incision was allowed to remain outside wrapped up in flannel	88 c.c. R.P. ·18 %= 0 gm. in 20 mins. 5·2 c.c. R.P. 1·6 %= ·083 gm. in 30 mins. 4·4 c.c. R.P. 3·1 %= ·136 gm. in 30 mins. S Total ·21 gm. in 1 hr. 20 mins.	—
12	4½ hrs. as previous	Section of symphysis pubis. Catheter tied into bladder. Peritoneal cavity not opened	? c.c. R.P. 1·19 % ? gm. in 30 mins. 4·5 c.c. R.P. 5·95 %= ·267 gm. in 1 hr.*	—
13	3·6 kilo. 4 hrs. as previous	Branch of s.m.a. in root of mesentery ligatured with disturbance of nerves	35 c.c. R.P. ·28 %= ·028 gm. in 25 mins. 17·6 c.c. R.P. 5·7 %= 1·003 gm. in 1½ hrs. S Total 1·03 gm. in 1 hr. 40 mins.	—
Experiments characterised by section of the great splanchnic nerves.				
14	3·85 kilo. Vomited after feeding this morning on milk with urethane dissolved in it	A.C.E. followed by ·35 gm. of urethane subcutaneously. Through incisions in back both gt. splanchnic nerves cut proximally of a ligature. Section of symphysis pubis, catheter tied into bladder Peritoneum opened. Branch of s.m.a. freed from adjoining nerves and ligatured	81 c.c. R.P. 1·59 %= 1·287 gm. in 2 hrs. 3·7 c.c. R.P. 7·14 %= ·264 gm. in 1½ hrs. 3·6 c.c. R.P. 2·64 %= ·095 gm. in 1½ hrs. Total 1·64 gm. in 4½ hrs.	Blood at end of exp. had R.P. ·167 %.
15	Both gt. spl. nerves cut 13 days previously. Fair condition. Exp. begun 2½ hrs. after milk	Section of symphysis pubis, catheter tied into bladder ... Branch of s.m.a. ligatured with disturbance of nerves... ... Animal died 10 mins. after injecting some blood-serum	R.P. of urine on day before exp. was <·2 %. 19 c.c. R.P. <·4 % = 145 gm. in 30 mins. 2·5 c.c. R.P. <·5 ·7 gm. in 90 mins. Total = ·7 91 gm. in 2 hrs.	Some minutes after death the liver contained glycogen 19·35%, sugar 11·5%.
16	2·9 kilo. Both gt. spl. nerves were cut 4 months previously. Good condition	Ligature of sp. art. and adjacent nerves. Ligature pulled upon three times in following hour	22 c.c. R.P. <·27 % = 0 gm. in 25 mins. 2·7 c.c. R.P. <1·5 % = ·04 gm. in 1½ hrs. S Total ·04 gm. in 2 hrs.	At end of exp. R.P. of carotid blood was ·159%. Liver 28·97% of glycogen.

TABLE XII. *Relation of the carbohydrate in the liver to the degree of glycosuria in cats.*

(All but very few of the animals had been fed up in the laboratory on meat, milk and carbohydrate for at least a fortnight, and had been fed on the morning of the experiment with meat and milk only, except those marked by an asterisk, indicating that about 20 gm. of cane-sugar were given dissolved in the milk four or more hours before the experiment.)

The *abbreviation* "L," used in the last column, indicates that there was slight loss in estimating the glycogen.

Number of exp.	Remarks	Quantity of urine and duration of experiment	Highest % of sugar found in urine	Total quantity of sugar excreted in exp. (gm.)	Carbohydrate in liver		
					Glycogen expressed as glucose. % of liver	Sugar expressed as glucose. % of liver	Total expressed as % of liver
α	Injection of 10 c.c. fasting dog's blood-serum. The liver estimation was made after incubating a portion of the minced liver with normal saline for 2½ hrs. at 38° C.	6 c.c. in 40 mins.	7·7	·21	16·8	30	46·8
Exp. 8	Table IX	11·2 c.c. in 1½ hrs.	5	·3	34	(3)	37
Exp. 16	Table XI	7·7 c.c. in 2 hrs.	1·5	·04	32·1	(3)	35·1
Exp. 15	Table XI	7·5 c.c. in 2 hrs.	<·5	·01	21·5	11·5	33
					The liver was not minced till 10 mins. after death		
β	Injection 27 c.c. thoracic lymph-serum fasting dog	6·7 c.c. in 1½ hrs.	1·3	·05	30·6	(3)	33·6
γ	Injection of 100 c.c. blood-serum of digesting dog. The second estimation was made after incubating a portion of the minced liver for 2½ hrs. at 38° C.	31 c.c. in 2½ hrs.	2·3	·71	28·7 7·4	4 21·9	32·7 29·3
*Exp. 6	Table XI	40·3 c.c. in 2 hrs.	2·2	·37	26·9	(3)	29·9
Exp. 17	Table X	22·8 c.c. in 2½ hrs.	1·8	·27	26·7	(3)	29·7
δ	Injection 50 c.c. blood-serum fasting dog ...	8·6 c.c. in 1½ hrs.	4·2	·17	26·1	(3)	29·1
Exp. 4	Table IX	13 c.c. in 1½ hrs.	6·6	·4	25·7	(3)	28·7
Exp. 12	Table IX	7 c.c. in 2 hrs.	6·2	·37	24·9	(3)	27·9
Exp. 11	Table IX	5·5 c.c. in 1½ hrs.	4·9	·1	24·7	(3)	27·7
Exp. 1	Table IX.	7·4 c.c. in 2 hrs.	3·6	·03	24·2	(3)	27·2

TABLE XI. (*Continued.*)

Number of exp.	Weight of cat. Last food	Experimental procedure	Quantity of urine and sugar excreted in experiment	Remarks
11	2·75 kilo. 5 hrs. after milk and meat (but no sugar)	It took 10 mins. to empty the bladder by squeezing. A small piece of the ileum which had escaped through incision was allowed to remain outside wrapped up in flannel	88 c.c. R.P. ·18 % = 0 gm. in 20 mins. 5·2 c.c. R.P. 1·6 % = ·083 gm. in 30 mins. 4·4 c.c. R.P. 3·1 % = ·136 gm. in 30 mins. S Total ·21 gm. in 1 hr. 20 mins.	—
12	4½ hrs. as previous	Section of symphysis pubis. Catheter tied into bladder. Peritoneal cavity not opened	? c.c. R.P. 1·19 % ? gm. in 30 mins. 4·5 c.c. R.P. 5·95 % = ·267 gm. in 1 hr.*	—
13	3·6 kilo. 4 hrs. as previous	Branch of s.m.a. in root of mesentery ligatured with disturbance of nerves	35 c.c. R.P. ·28 % = ·028 gm. in 25 mins. 17·6 c.c. R.P. 5·7 % = 1·003 gm. in 1½ hrs. S Total 1·03 gm. in 1 hr. 40 mins.	—
Experiments characterised by section of the great splanchnic nerves.				
14	3·85 kilo. Vomited after feeding this morning on milk with urethane dissolved in it	A.C.E. followed by ·35 gm. of urethane subcutaneously. Through incisions in back both gt. splanchnic nerves cut proximally of a ligature. Section of symphysis pubis, catheter tied into bladder Peritoneum opened. Branch of s.m.a. freed from adjoining nerves and ligatured	81 c.c. R.P. 1·59 % = 1·287 gm. in 2 hrs. 3·7 c.c. R.P. 7·14 % = ·264 gm. in 1½ hrs. 3·6 c.c. R.P. 2·64 % = ·095 gm. in 1½ hrs. Total 1·64 gm. in 4½ hrs.	Blood at end of exp. had R.P. ·167 %.
15	Both gt. spl. nerves cut 13 days previously. Fair condition. Exp. begun 2½ hrs. after milk	Section of symphysis pubis, catheter tied into bladder ... Branch of s.m.a. ligatured with disturbance of nerves... ... Animal died 10 mins. after injecting some blood-serum	R.P. of urine on day before exp. was < ·2 %. 19 c.c. R.P. < ·4 % = ·008 gm. in 30 mins. 2·5 c.c. R.P. < ·5 % = ·007 gm. in 90 mins. Total ·01 gm. in 2 hrs.	Some minutes after death the liver contained glycogen 19·35%.sugar11·5%.
16	2·9 kilo. Both gt. spl. nerves were cut 4 months previously. Good condition	Ligature of sp. art. and adjacent nerves. Ligature pulled upon three times in following hour	22 c.c. R.P. < ·27 % = 0 gm. in 25 mins. 2·7 c.c. R.P. < 1·5 % = ·04 gm. in 1½ hrs. S Total ·04 gm. in 2 hrs.	At end of exp. R.P. of carotid blood was ·159%. Liver 28·97% of glycogen.

TABLE XII. *Relation of the carbohydrate in the liver to the degree of glycosuria in cats.*

(All but very few of the animals had been fed up in the laboratory on meat, milk and carbohydrate for at least a fortnight, and had been fed on the morning of the experiment with meat and milk only, except those marked by an asterisk, indicating that about 20 gm. of cane-sugar were given dissolved in the milk four or more hours before the experiment.)

The *abbreviation* "L," used in the last column, indicates that there was slight loss in estimating the glycogen.

Number of exp.	Remarks	Quantity of urine and duration of experiment	Highest % of sugar found in urine	Total quantity of sugar excreted in exp. (gm.)	Carbohydrate in liver		
					Glycogen expressed as glucose. % of liver	Sugar expressed as glucose. % of liver	Total expressed as % of liver
α	Injection of 10 c.c. fasting dog's blood-serum. The liver estimation was made after incubating a portion of the minced liver with normal saline for 2½ hrs. at 38° C.	6 c.c. in 40 mins.	7·7	·21	16·8	30	46·8
Exp. 8	Table IX	11·2 c.c. in 1½ hrs.	5	·3	34	(3)	37
Exp. 16	Table XI	7·7 c.c. in 2 hrs.	1·5	·04	32·1	(3)	35·1
Exp. 15	Table XI	7·5 c.c. in 2 hrs.	<·5	·01	21·5	11·5	33
					The liver was not minced till 10 mins. after death		
β	Injection 27 c.c. thoracic lymph-serum fasting dog	6·7 c.c. in 1½ hrs.	1·3	·05	30·6	(3)	33·6
γ	Injection of 100 c.c. blood-serum of digesting dog. The second estimation was made after incubating a portion of the minced liver for 2½ hrs. at 38° C.	31 c.c. in 2½ hrs.	2·3	·71	28·7 7·4	4 21·9	32·7 29·3
*Exp. 6	Table XI	40·3 c.c. in 2 hrs.	2·2	·37	26·9	(3)	29·9
Exp. 17	Table X	22·8 c.c. in 2½ hrs.	1·8	·27	26·7	(3)	29·7
δ	Injection 50 c.c. blood-serum fasting dog ...	8·6 c.c. in 1½ hrs.	4·2	·17	26·1	(3)	29·1
Exp. 4	Table IX	13 c.c. in 1½ hrs.	6·6	·4	25·7	(3)	28·7
Exp. 12	Table IX	7 c.c. in 2 hrs.	6·2	·37	24·9	(3)	27·9
Exp. 11	Table IX	5·5 c.c. in 1½ hrs.	4·9	·1	24·7	(3)	27·7
Exp. 1	Table IX.	7·4 c.c. in 2 hrs.	3·6	·0	24·2	(3)	27·2

TABLE XII. (*Continued.*)

Number of exp.	Remarks	Quantity of urine and duration of experiment	Highest % of sugar found in urine	Total quantity of sugar excreted in exp. (gm.)	Carbohydrate in liver: Glycogen expressed as glucose. % of liver	Carbohydrate in liver: Sugar expressed as glucose. % of liver	Carbohydrate in liver: Total expressed as % of liver
ε	Injection 50 c.c. blood-serum digesting dog. Cat died from bronchial obstruction due to anæsthetic catarrh	7·6 c.c. in 2½ hrs.	·4	·04	14·9	12	26·9
					The liver was not minced till about 8 mins. after death		
*Exp. 5	Table XI	33·5 c.c. in 2½ hrs.	5	1·32	21·9	(3)	24·9
Exp. 15	Table X	12·9 c.c. in 2½ hrs.	5·7	·54	19·6	5·1	24·7
Exp. 10	Table IX	12·2 c.c. in 1½ hrs.	8·5	·71	21·3	(3)	24·3
ζ	Injection 90 c.c. saline extract of sheep's lymph-glands	13·2 c.c. in 2½ hrs.	5·2	·3	19·5	(3)	22·5
Exp. 16	Table X	29·2 c.c. in 2¾ hrs.	4·3	·6	18·5	(3)	21·5
Exp. 8	Table IX	10·5 c.c. in 2 hrs.	·3	0	17·6	(3)	20·6
Exp. 18	Table X	5·5 c.c. in 1½ hrs.	·8	·03	17·3	(3)	20·3
Exp. 8	Table XI	33 c.c. in 2 hrs.	1·4	·47	17·2	(3)	20·2
Exp. 11	Table X	22·1 c.c. in 3¾ hrs.	8·6	·73	15·9	(3)	18·9 L
Exp. 9	Table IX	14·1 c.c. in 2 hrs.	5·8	·53	15·1	(3)	18·1
Exp. 2	Table IX	5·6 c.c. in 2½ hrs.	4·7	·26	15	(3)	18
η	Injection 50 c.c. th. lymph-serum fasting dog. The second estimation was made after incubating a portion of the liver for 2 hrs. at 43° C.	7 c.c. in 3½ hrs.	8·2	·21	13·4 2·5	4·5 14	17·9 16·5
θ	Injection 20 c.c. th. lymph-serum fasting dog. Spleen excised	14·4 c.c. in 1 hr.	4	·66	2·7	15	17·7 L
					The liver was not estimated till 2½ hrs. after death		
Exp. 1	Table XI	11·4 c.c. in 2½ hrs.	< ·6	< ·07	11·6 4·6	2·7 12·2	14·3 L 16·8
Exp. 14	Table IX	12·3 c.c. in 1½ hrs.	1·8	·12	13·4	(3)	16·4
ι	Injection 40 c.c. th. lymph-serum fasting dog. Splenic art. and nerves ligatured	10·8 c.c. in 1½ hrs.	3·2	·2	13	(3)	16

Exp. 13	Table IX	6 c.c. in 1½ hrs.	·3	·02	12·3	(3)	15·3
Exp. 7	Table IX	12·8 c.c. in 2 hrs.	·4	·04	11	(3)	14
*Exp. 4	Table XI	26 c.c. in 1⅔ hrs.	5	1·19	10·7	(3)	18·7
*Exp. 8	Table XI	21·7 c.c. in 1 hr.	4·1	·85	10·4	(3)	18·4
Exp. 15	Table IX	8·1 c.c. in 1½ hrs.	1·2	·06	10·3	(3)	13·3
Exp. 18	Table X	11 c.c. in 3½ hrs.	11·9	·86	9·8	(3)	12·8
Exp. 5	Table IX	20 c.c. in 2 hrs.	1	·31	9·2	(3)	12·2
κ	Injection 50 c.c. th. lymph-serum fasting dog. Splenic art. and nerves ligatured	10·4 c.c. in 2½ hrs.	·8	·05	2·8	7·7	10·5
						The liver was not estimated till 30 mins. after death	
λ	Injection 55 c.c. blood-serum fasting dog ...	12 c.c. in 1½ hrs.	3·1	·39	7·2	(3)	10·2
Exp. 2	Table XI	41·5 c.c. in 2½ hrs.	·4	< ·08	5·4	(3)	8·4
μ	Injection 60 c.c. th. lymph-serum fasting dog into saphenous vein. Splenic art. and nerves ligatured	37·7 c.c. in 3½ hrs.	2·6	1·04	4·1	(3)	7·1
ν	Injection 60 c.c. th. lymph-serum fasting dog. Splenic art. and nerves ligatured	25 c.c. in 2⅔ hrs.	1·7	·25	3·5	(3)	6·5
ξ	Injection 120 c.c. blood-serum fasting dog. This estimation of liver was made after incubating a portion with lymph at 38° C. for 1½ hrs.	9·1 c.c. in 3 hrs.	·7	·07	2·2	3·8	6
ο	Injection 35 c.c. th. lymph-serum fasting dog. The 2nd estimation made after incubating a portion of liver with normal saline at 44° C. for 2 hrs.	9·3 c.c. in 2½ hrs.	3·6	·15	2·9 1·9	2·6 3	5·5 4·9
Exp. 14	Table X	8·7 c.c. in 2½ hrs.	·4	·02	2·7	2·3	5
Exp. 6	Table IX	6·9 c.c. in 2 hrs.	2·3	·06	1·4	(3)	4·4

All injections were made into the splenic vein except in μ. The anæsthetic used in all cases was A.C.E. followed by ether given through a trachea-tube.

In the majority of the experiments the sugar in the liver was not estimated so that the figure "3" enclosed in brackets represents only approximately the quantity of sugar in the liver, based on the results obtained in the few cases where the hepatic sugar was estimated.

In calculating the quantity of urine, I have arbitrarily assumed that urine was secreted between the beginning of the experiment and the time of collecting the first urine, at the rate of 5 c.c. in 30 mins. in cases where I found the bladder very full and obviously containing much more urine than could have been secreted since the commencement of the experiment.

breathing well and showing no signs of asphyxia or anæsthetic trouble, to be 3 %, as that is approximately the figure I have found in the few cases where I have made an estimate of the sugar in the alcoholic extract as well. In this table the glycogen is expressed in terms of glucose. The sugar in the alcoholic filtrate was estimated as follows [and the description applies equally well to my method of estimating sugar in the blood, received from the carotid artery of an animal into a weighed quantity of alcohol:—see in particular the experiments recorded in Table IX]:

The alcoholic solution was placed in a closed glass vessel, with a side arm, through which the air was exhausted, while the vessel was placed in a water bath at a temperature of about 60° C. In a few hours the alcohol had evaporated away and the dry residue I then dissolved in boiling water, using alumina for decolourising the material if necessary. The aqueous solution was then filtered and, after most carefully washing out any sugar in the insoluble residue on the filter paper (which was reliably free from any soluble carbohydrate), I then made it up to known bulk and estimated with Pavy's fluid, both before and after inversion [boiling for an hour under a reflux condenser with that quantity of HCl which made it contain 2·2 % of the acid].

As will be seen from Table XII (on comparing columns 5 and 8), there is no exact proportion between the two sets of figures, but in cases where the total quantity of carbohydrate is less than 10 % the amount of sugar excreted is usually less than ·4 gm. in about 2 hrs. However, there are notable exceptions in both directions. Thus in Exp. μ over 1 gm. of glucose was excreted, although only 7·1 % was found in the liver, while in Exp. 3 (Table IX) no sugar was excreted although the liver contained as much as 20 %. It may of course be questioned whether the factors making for sugar-production (anæsthetic and disturbance of nerves) were equally strong in the two experiments and it might be suggested that where the experimental conditions did not lead to pronounced sugar-formation in the liver, there would be found associated together considerable glycogen in the liver and minimal glycosuria; while pronounced glycosuria might lead to the emptying of the liver. Thus in Exp. α, where I found the maximum amount of hepatic carbohydrate, I only obtained an excretion of ·21 gm. of glucose; but then the experiment only lasted 40 mins. because the animal died during the experiment, so that, if the experiment had been continued, I might have got a very different result, as the glycosuria had already reached the high level of 7·7 %. On the other hand in Exps. 15 and 16

(Table XI), where both great splanchnic nerves had been cut previously and so nervous impulses to the liver had been cut off, I found respectively 33 % and 35 % of carbohydrate in the liver, while only ·01 and ·04 gm. of glucose came out in the urine.

Another factor which makes for uncertainty is the renal sensitiveness in excreting sugar. Thus I am certain that experiments like η, ξ, 18 (Table X) and 2 (Table IX), where a small quantity of urine was excreted, sometimes containing a high percentage of sugar [in Exp. 18 (Table X) as high as 12 %], are not comparable with experiments like 2 and 6 (Table XI) where a large quantity of urine was secreted. In fact the relation between hyperglycæmia and the percentage of sugar in the urine is far from simple, a point to which I shall return at the end of this paper.

The only factor in these experiments on cats—apart from Nos. 15 and 16 (Table XI)—which seems certainly to have affected the result, is the nature of the last meal. If sugar is given, it tends to appear in the urine. Thus the most striking feature about Table XI is the amount of sugar excreted in Exps. 4, 5, 6, 7, 8, 9 and 10 (Table XI), where approximately 20 gm. of cane-sugar were given dissolved in milk from 4½ (Exp. 10) to 8¾ hours (Exp. 7) before the commencement of the experiment. This amount of sugar must have been absorbed and presumably metabolised some time before the commencement of the experiment and yet in No. 10 as much sugar as 5 gm. was excreted in the urine. In such experiments of course the relation between the sugar excreted in the urine and the carbohydrate found in the liver is markedly different from what I found in most of my other experiments.

This reappearance of sugar from the last meal suggests some inability on the part of the liver to store it as glycogen, and recalls the old experiments of Seelig[39] and Naunyn[30], who respectively found (*a*) that if 2 gm. of glucose was injected into the jugular vein of a normal fasting rabbit, ·2 gm. of glucose, as the mean of many experiments, were excreted in the urine, whereas after diabetic puncture the mean amount was found to be ·6 gm. or three times as much; (*b*) that if glucose was injected into the mesenteric vein of a healthy normal dog, the liver of which contained no glycogen, very little sugar appeared in the urine, whereas after diabetic puncture a large quantity was excreted.

Now although perhaps Table XII supports on the whole the view that the glycosuria of anæsthetic and experimental conditions comes

from the liver, I think I should here point out some of the latest work concerning the relations of hepatic glycogen to other possible sources of sugar. Pflüger[35] for many years has advocated the view that sugar comes from fat, while Blum[36] showed that on a fatty diet a much better adrenine-glycosuria could be produced than in hunger. Velich[37] again, found in frogs that adrenine produced no glycosuria in those animals in which the "fat-bodies" had been previously removed. Likewise Eppinger, Falta and Rudinger[38] found (*a*) that adrenine-glycosuria was much increased by a fatty diet being given to dogs, which had previously been made practically glycogen-free through muscular work and fasting; (*b*) that in fasting dogs at the height of the disturbance following excision of the pancreas (when the liver certainly contains no glycogen), the quotient $\frac{D}{N}$ can transitorily be made to rise through adrenine, implying the formation of sugar from fat.

Thus adrenine appears to have the effect of accelerating what Falta calls the "mobilisation (Mobiliserung) of fat and carbohydrate in the body." Therefore I am inclined to come to the conclusion about the source of the sugar-production in all my experiments (morphine and ether-narcosis alone or associated with fistula of the thoracic duct in dogs and with disturbance of visceral nerves in cats), that, while the glycogen of the liver must take an important part in the "mobilisation" of carbohydrate in the body, yet it is quite likely that this is only an indirect effect, the result of other factors, such as the hyper-production of adrenalin accelerating fat and carbohydrate transformation throughout the body. Such a view has already been put forward to explain the effects following damage to the floor of the IVth ventricle of the brain; for Blum considered that the glycosuria of diabetic puncture was related to adrenine glycosuria, the nervous impulses taking the course discovered by Claude Bernard, viz., the great splanchnic nerves, but going to the supra-renal capsules and only indirectly causing a conversion of hepatic glycogen into sugar through hyper-production of adrenine. I have myself done only three experiments on cats with the great splanchnic nerves cut, and consequently they are too few in number to prove much; yet they are suggestive as far as they go. In Exp. 14 (Table XI), in which the great splanchnic nerves were cut in the course of the experiment, subsequent disturbance of the nerves running with the superior mesenteric artery was associated with a definite, though not considerable, glycosuria; but then the disturbance set up by anæsthetisation and

section of the great splanchnic nerves had already produced a marked excretion of sugar, amounting to 1·28 gm. in 2 hours. In Exp. 15, in which the nerves had been cut 13 days before, there was practically no glycosuria in 2 hours following anæsthetisation, section of the symphysis pubis, tying a catheter into the bladder and disturbance of nerves running with a branch of the superior mesenteric artery, although the liver contained 33 % of carbohydrate reckoned as glucose. In Exp. 16, in which both nerves had been cut 4 months before, there was slight glycosuria (·04 gm. in 1½ hrs.) following anæsthetisation and ligature of the splenic artery and contiguous nerves, and the percentage of sugar in the urine rose to 1·5 %. Here also the liver contained from 32 % to 35 % of carbohydrate.

Recently, also, the work of Mottram[89] has proved that the liver in a state of fast has the power of attracting to itself fat from the fatty store-houses of the body. Now, seeing that the liver has such important functions to perform, this looks as if the liver tries to do its work by utilising the energy derivable from the metabolism of fat; and, as an important function of the liver is undoubtedly that of regulating the supply of carbohydrate to the muscles—as proved by the classical experiments of Külz[90] on the influence of exhausting exercise in dogs—a very probable deduction from these facts is the view that the liver has the power of producing sugar from fat as well as from glycogen and that, in the absence of a store of glycogen, some power of sugar-production (from fat) is still possessed by the liver.

... ...

Hyperglycæmia and Glycosuria.

Before concluding this paper a few words about the relation of sugar in the urine to sugar in the blood may not be out of place, as I have made a number of sugar-analyses of the blood in my experiments on cats. The results are given in Table XIII. In all the experiments quoted there the blood was received from a cannula in the carotid artery straight into a weighed quantity of alcohol, so that no post-morten changes could occur; and there was no chance of any sugar from the liver affecting the result, as sometimes, I believe, may occur when the blood is taken from the heart.

In every case the blood was taken at the end of an experiment, when the percentage of sugar in the urine was usually much lower than it had been previously, though occasionally, as in Exp. 11 (Table IX), the end of the experiment was attended by a high degree of glycosuria.

TABLE XIII. *Relation of glycosuria to hyperglycæmia in cats.*

[The last column shows the time between the penultimate collection of urine and taking of blood at end of Exp.]

Number and nature of experiment	R.P. of blood before and after inversion		Urine collected at end of experiment		
	%	%	Quantity c.c.	R.P. %	Time of secretion mins.
1. Table IX	·206	·207	6·1	< ·6	90
2. ,, ...	·224	—	4·4	4·7	60
3. ,, ...	·16	·158	2·7	·3	70
4. ,, ...	·23	·21	3·1	4·2	50
7. ,, ...	·23	·24	2·4	·4	50
8. ,, ...	·131	·135	2·7	·34	60
9. ,, ...	·211	·203	4·9	6·2	78
10. ,, ...	·184	·195	2	1·31	70
11. ,, ...	·214	—	6·5	8·54	68
12. ,, ...	·158	·167	2·2	1·35	50
13. ,, ...	·174	·163	2·6	2·95	45
14. ,, ...	·17	·17	6·5	1·58	65
15. ,, ...	·164	—	4·3	1·24	60
16. ,, ...	·174	·167	8	1·58	90
14. Table X	·18	·19	·6	< ·9	75
15. ,, ...	·26	·28	5·2	4·7	40
16. ,, ...	·32	·33	9·7	4·3	65
17. ,, ...	·18	·19	4	·5	15
18. ,, ...	·23	·22	1·2	3·4	100
14. Table XI	·16	·16	3·6	2·6	95
16. ,, ...	·16	·16	2·7	1·5	90
β. Table XII	·18	·18	3·3	1·3	60
ι. ,, ...	·24	·23	2·3	3	30
μ. ,, ...	·21	·22	6·5	2·5	35
ρ. ,, ...	·18	·17	9·5	·6	70
τ. Under Chlorotone, A.C.E. and Ether the s.m.s. was ligatured, and catheter tied into bladder.	·14	·15	4	·4	120

In this experiment, in which 6½ c.c. of urine were secreted during the hour previous to taking the blood, the kidneys may be considered to have been secreting fairly well and here we see that 8·5 % of sugar in the urine corresponded to ·21 % of sugar in the blood. In Exp. 18 (Table X), on the other hand, where the kidneys were certainly secreting badly as only 1·2 c.c. of urine were secreted during 1 hr. 40 mins. previous to taking the blood, ·23 % of sugar in the blood corresponded to as little as 3·4 % of sugar in the urine, although at an earlier period of this experiment the sugar in 1·4 c.c. of urine amounted to the very high figure of 13 %. Exps. 1 (Table IX) and 16 (Table X) were also

attended by a lower degree of glycosuria than one would have expected from the degree of hyperglycæmia. Again, if experiments with similar hyperglycæmia figures are compared, it will be found that sometimes the urine contained a higher percentage of sugar in those experiments in which least urine was secreted. Thus if Exp. μ be compared with Exp. 9 (Table IX), it will be seen that while in the former 6·5 c.c. were secreted in 35 mins. and contained 2·5 % of sugar, the latter (yielding the same degree of hyperglycæmia, viz. 21 %), was characterised by a secretion of 4·9 c.c. urine in 78 mins. with an R.P. of 6·2 %. Similarly Exps. 13, 14, 16 (Table IX), all yielding a hyperglycæmia figure of 17 %, were attended by a higher degree of glycosuria, the smaller the quantity of urine secreted.

Consequently from the percentage of sugar in the urine it is very difficult to say with any certainty what the corresponding degree of hyperglycæmia would be. Nevertheless it may be stated from the results given by these experiments—as a general rule with some exceptions—that anything over ·2 % of hyperglycæmia was associated with marked glycosuria (2 % and upwards), while ·18 % hyperglycæmia was the figure which corresponded to a slight glycosuria and ·15 % hyperglycæmia might be associated with little or no glycosuria.

Apart from the question of the kidneys secreting well or badly, the important factor seems to be, whether the sugar in the blood is rising or falling in amount. If it is rising, it may of course up to that point have been unable to give rise to any noticeable glycosuria, whereas, if it is falling, the hyperglycæmia will probably be found smaller than might be expected from the degree of glycosuria. That is how I should be inclined to explain an experiment like No. 16 (Table X), where the kidneys appear to have been secreting well and yet the urine at the end only contained 4·3 % of sugar with a hyperglycæmia figure of ·32 %.

I have only two experiments in dogs with reliable figures for the hyperglycæmia caused by morphine narcosis at the height of the disturbance. These gave the following results:

Exp. O 3 [Table VII]. The blood was taken by a cannula in the carotid artery 1 hr. 20 mins. after the injection of morphine. This dog possessed only a fragment of pancreas.

Urine taken 35 mins. before taking blood		R.P. of blood	Urine taken 10 mins. after taking blood	
Quantity	R.P.		Quantity	R.P.
13 c.c.	6·8 %	·26 %	20 c.c.	10·7 %

Exp. X [Table VIII]. The blood was taken by a cannula in the carotid artery 2¾ hrs. after the injection of morphine and 1¾ hrs. after a fistula of the thoracic duct had been established.

Urine taken 30 mins. before taking blood		R.P. of blood	Urine taken 30 mins. after taking blood	
Quantity	R.P.		Quantity	R.P.
10·5 c.c.	7·9 %	·26 %	6 c.c.	5·6 %

In O 3 the kidneys were secreting better than in X and thus the degree of hyperglycæmia was as high in the latter as in the former, although the percentage of sugar in the urine was rising in the one case and falling in the other. But the two experiments show fairly well that in the glycosuria following morphine-narcosis 7 % of sugar in the urine corresponds roughly to ·26 % of sugar in the blood.

There is one other point which comes out quite clearly from the experiments recorded in Table XIII, namely that the sugar in the blood at the moment of drawing has an R.P. not increased by inversion. Therefore the sugar in the blood of these cats subjected to anæsthetisation and disturbance of viscera, is not quite the same as the sugar in normal cat's blood; for Pavy[10] found the R.P. of normal cat's blood was increased by inversion to an extent represented by the proportion 77 : 100—(in a normal rabbit 73 : 100). Possibly the method of drawing the blood has some influence. For Pavy took his blood from the still beating heart; and similarly I found the R.P. of the blood-sugar was increased by inversion in some of my earlier experiments, in which I took the blood from an incision in the heart and estimated the blood serum after whipping and filtering the clot away through muslin.

Conclusions.

The following then is a summary of the conclusions which may be drawn from my experiments:

(1) There is no evidence that inhibition of the internal secretion of the pancreas is a factor in the production of anæsthetic and experimental glycosuria; nor is there any evidence in favour of the view that carbohydrate in the diet stimulates the pancreas to increased production of its internal secretion.

(2) The glycosuria following fistula or ligature of the thoracic duct in dogs is a real phenomenon; but there is no evidence to prove that it is brought about by other factors than the anæsthetic and disturbance of nerves. At the same time if the internal secretion of the pancreas

finds its way into the circulation by way of the thoracic duct, it is possible that this might be a subsidiary factor in the production of the glycosuria.

(3) The glycosuria associated with morphine and ether-narcosis with or without disturbance of nerves is not caused by a diminished consumption of sugar in the body owing to a deficiency of the internal secretion of the pancreas; but is the result of a rapid production of sugar, not only from the glycogen of the liver but also from other sources of carbohydrate in the body, including fat. This production of sugar may be caused indirectly by the action of chemical bodies like adrenine. Consequently the glycosuria studied in this thesis is of a type resembling diabetes mellitus in man rather than pancreatic diabetes in dogs. For, as Falta[13] has proved, there are marked differences between the two states and in some cases of very severe diabetes mellitus with enormous hyper-production of sugar there is still some power of consuming sugar left.

(4) In "experimental glycosuria" the relation between the degree of glycosuria and hyperglycæmia is variable: but the sugar in the blood of cats, exhibiting "experimental glycosuria," has a reducing power equal to that of dextrose.

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THE SECRETION OF URINE. By JOSEPH BARCROFT and HERMANN STRAUB.

(*From the Physiological Laboratory, Cambridge.*)

The researches of Schlayer[1], his co-workers and others have made it desirable that some further investigation of the metabolism of the kidney during diuresis, should be made. Schlayer recognises two kinds of diuresis, the mechanism of the one is vascular and the other tubular. The action of the vascular diuretics is maintained after the cells of the tubules have been poisoned with salts of the heavy metals. The original intention of our research was to investigate the vascular diuretics for the purpose of finding out whether, or no, their action was associated with increased metabolism of the kidney, and if so whether such had its seat in the glomerular epithelium.

Heretofore the only diuretics whose action on the mammalian kidney[2] has been studied in this way are urea, sodium sulphate and phloridzin. There is a considerable amount of evidence culled from various organs in the body—striped[3], and cardiac muscle[4], submaxillary[5] and parotid glands[6], pancreas[7], kidney with certain diuretics[8], intestine[9]—which leads us to suppose that activity of the organ is associated with an immediate increase in the oxygen taken up by it, assuming that the oxygen is available, and we have regarded it as a fair assumption that if urine appears without increased oxidation in the kidney itself, the mechanism which accounts for its appearance is a purely physical one, whereas if there is a well marked increase in the metabolism of the kidney a secretory process is called into play.

General method of experiment. Our experiments have been performed on cats and rabbits; dogs proved unsuitable, because the large doses of diuretic necessary were retained in the tissues to such an extent that it was found impossible to free the animal from them. The dissections do not differ materially from those described by

Barcroft and Brodie[8]. The small amount of blood which comes from the cat's kidney (and the still smaller amount from the rabbit's) makes it possible to use the method of collection and of measurement of rate of flow described by one of us for the investigation of the salivary glands[10]. When possible we used but one kidney and in some cases put our venous cannula into the ovarian vein—a very convenient arrangement when this vein is large enough.

The oxygen analyses have been made by the differential method[11]. Blood samples of 1 c.c. or 2 c.c. each have been analysed. The blood has been kept from clotting by the use of a trace of hirudin[12] in the collecting tubes.

The anæsthetic, unless otherwise stated, has been urethane.

Diuresis evoked by Ringer's solution.

In our first experiments the diuretic used was a solution of the following composition, NaCl ·9%, KCl ·042%, CaCl, ·024%, Na_2CO_3 ·03%. The diuresis evoked by this solution will be called throughout "normal saline diuresis." Isotonic sodium chloride solution has been used by several observers, *e.g.* Magnus[13] and Frey[14]. Injected into rabbits in large quantities, up to the total body weight of the animal, it has been found to produce an immediate and considerable diuresis. This phenomenon is, of course, distinct from that observed by Thompson (This *Journal*, xxv. p. 487). We have obtained a good flow of urine with much smaller injections than they describe. This may be due directly or indirectly to the fact that our animals were eviscerated, for they had a good blood-pressure and small blood volume and there was no opportunity of their water being excreted into the intestinal tract.

Whilst injection of Ringer's solution, into the jugular vein of a cat in good condition, very shortly produces a flow of urine, it produces no increase, and what is equally important no decrease in the oxidation of the kidney. Before we can accept this statement as a valid proof of a mechanical production of urine it is necessary to consider several questions.

(1) Is the "normal saline" diuresis due to injury of the kidney? In order to settle this question we have controlled the diuresis as produced by Ringer's solution with a sodium sulphate diuresis before and after. Sodium sulphate is known to cause increased oxidation[15] in the kidney. The general course of such an experiment (Exp. 1) may be seen in Fig. 1 in which the oxygen used by the kidney and the volume

of urine are plotted vertically and the time horizontally. The points on which we would insist are:

(*a*) During the sodium sulphate diuresis, at the beginning and at the end of the experiment there was a great rise of the oxygen taken.

(*b*) During the "normal saline diuresis," which was greater and more prolonged than that produced by sodium sulphate, there was no corresponding rise.

(*c*) The kidney, in the matter of oxidation, responded as well to the sodium sulphate at the end as at the beginning of the experiment.

(*d*) The urine did not contain either albumen or hæmoglobin at any stage of the experiment.

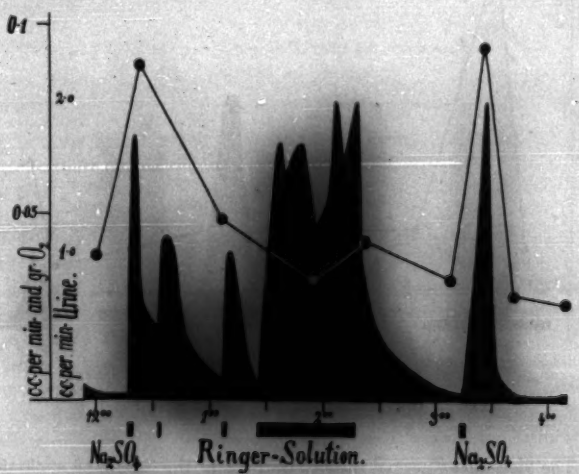

Fig. 1. Cat. The black line represents the oxygen taken up by the kidney in c.c. per gram of kidney per minute, the black areas represent the volume of urine secreted; urine per minute being plotted vertically and the time horizontally.

In Exp. 2 (Fig. 2) the above statements are confirmed in every respect save that the initial oxidation with sodium sulphate was greater than the final one, a fact which is reflected in the composition of the urine secreted. In the first diuresis the chlorides (reckoned as NaCl) were ·17 % and the sulphates (reckoned as Na_2SO) were 2·3 %, the urea ·5 %;

in the second sulphate diuresis the chlorides were ·42 and the sulphates 1·25 and the urea ·25 %; clearly in the second case the urine approximated more to the plasma than in the first.

(2) Is "normal saline" diuresis due to inhibition? Such for instance as might be supposed to take place if the reabsorptive function of cells of the tubules were abolished? The answer to this question is negative, since the metabolism of the kidney is not reduced.

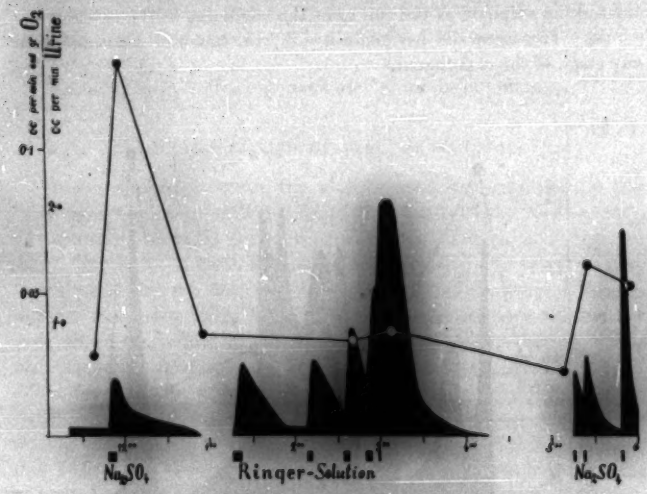

Fig. 2. Cat. Oxygen and urine plotted as in Fig. 1.

The view that "normal saline" diuresis is the result of a physical process which does not entail any increased output of energy on the part of the tubules, is in harmony with the nature of the urine itself. The sulphate diuresis in each case was marked by an amount of sulphate in the urine which was much greater, and an amount of chloride which was much less, than that of the plasma, whilst in the "normal saline" diuresis the salts in the urine are very like those of the plasma in composition. The following table will illustrate this point—for purposes of comparison we give also the salts in the urine which was collected from the bladder prior to the operation.

Salts in cat's urine. Exp. 1.

	Bladder prior to operation	1st sulphate diuresis	Ringer diuresis	2nd sulphate diuresis
Chlorides as NaCl	·42 %	·22 %	·86 %	·39 %
Sulphates as Na_2SO_4	·26 %	1·25 %*	·38 %	2·0 %
Urea	4·4 %	·28 %	·15 %	·25 %
Oxygen used up per grm.	0·4 c.c.	0·9 c.c.	0·3 c.c. 0·4 c.c.	0·9 c.c.

* At close of diuresis.

In confirmation of the observation that there is no increase in the oxygen used up by the kidney during "normal saline" diuresis we give the following data from other experiments.

Oxygen used up per gram of kidney per minute, and urine secreted per minute.

		Before injection	During Ringer diuresis			After
Exp. 3.	Oxygen	0·03	0·033	0·030	0·026	—
	Urine	0·1	0·4	0·5	0·4	—
Exp. 4.	Oxygen	0·10	0·11	0·11	—	0·09
	Urine	0·06	2·3	0·6	—	0·1
Exp. 5.	Oxygen	0·11	0·10	—	—	—
	Urine	·02	0·9	—	—	—

Sodium chloride. The transition from Ringer's solution to hypertonic and hypotonic solutions of sodium chloride was the natural extension of the above experiments. The case of hypotonic sodium chloride is quite clear, namely it consists in a physical production of urine without the aid of work done by the kidney. This is illustrated in Exp. 6 (Fig. 4) in which there was a considerable diuresis amounting to 1 c.c. of urine per minute, the oxygen used before the diuresis was ·73, during the diuresis ·71 and afterwards ·68 c.c. per gram per minute. As confirmatory of this result is one which was obtained at the end of Exp. 7 (Fig. 3), the oxygen taken in during the water diuresis was ·94 c.c. per gram per minute; this was preceded by an estimation of ·93 when the urine secreted was not more than a twentieth part of what it was during the diuresis: there was however a slight but appreciable fall after the diuresis to ·66 c.c. of oxygen.

In the case of hypertonic salt solutions diuresis does not seem to be the result of an active secretion. The most copious diuresis which we have obtained in the cat has resulted from the injection of 5 c.c. of 5 %

sodium chloride. In Fig. 3, it has been necessary to shorten the height of the record by cutting out a portion. The quantity of urine reached 5 c.c. per minute whilst before the diuresis the kidney was secreting only 0·04 c.c., nevertheless the oxygen used up in the normal period was 0·81 c.c. per gram per min. and during the diuretic period 0·83. About half an hour subsequently, the diuresis having by that time passed off, the metabolism seemed to have risen slightly, viz. to 0·93 c.c. Whether this rise was due to some stimulating effect of the chloride, or whether it was adventitious, we were not in a position to say.

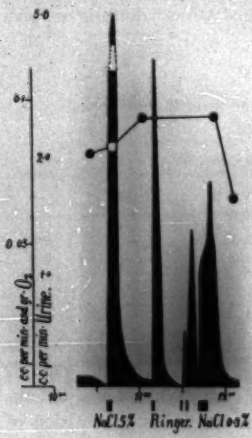

Fig. 3. Cat.

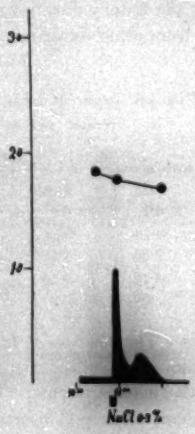

Fig. 4. Cat.

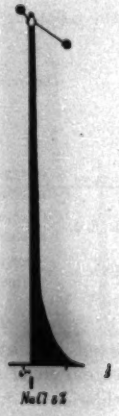

Fig. 5. Rabbit.

In Fig. 3 the column representing the first diuresis has been shortened, the apex being at 5 c.c. The apex of Fig. 5 is 3 c.c.

We therefore performed Exp. 8 shown in Fig. 5 which gave no rise either during or after the diuresis.

Caffeine and urea. The effects produced by caffeine and urea are somewhat similar to one another, but different from those already studied. Both these substances appear to cause a rise in the metabolism during the period of diuresis, followed by a fall of the metabolism to an abnormally low level. This depressing or poisoning action has been figured by Barcroft and Brodie in the case of urea, though its significance was not appreciated by them (*Journ. of Physiol.* XXXIII.

p. 61, Fig. 2, Exp. 6). In the single experiment with urea, a record of which appears in the present paper (Exp. 9, Fig. 6), the metabolism rose during the extended period of diuresis, then fell to about $\frac{2}{3}$ of its original level and subsequently recovered completely from the depression caused by the first dose of urea (10 c.c. of 5 %). A second and very large dose, 5 c.c. of 80 % urea, produced a still further depression from which the recovery was but very slight.

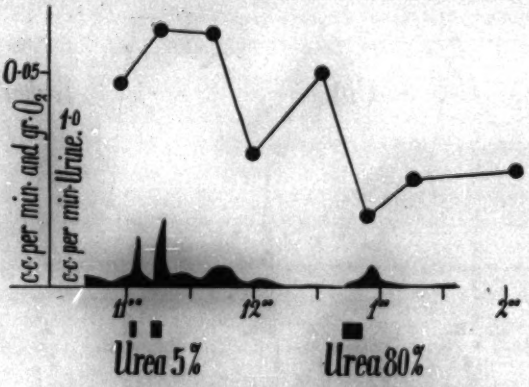

Fig. 6. Cat. Oxygen and urine plotted as in Fig. 1.

Two experiments (10 and 11, Figs. 7 and 8) have been performed with caffeine, one in the cat and the other in the rabbit. In both the same general features may be seen—namely (1) the stimulating effect of the drug during the diuretic period, (2) the subsequent depressing effect. In the case of the second dose there was a still further depressant effect in both animals, though with this difference: in the cat the second dose caused no diuresis, the depression set in at once; in the rabbit, which is notoriously tolerant to caffeine, the second dose caused a small diuresis, and with it the metabolism was augmented temporarily. The doses of caffeine used in these experiments were as follows. In the rabbit (weight 2 kilos) the first dose was 2 c.c. of 5 % caffeine sodium benzoate solution in 10 c.c. of defibrinated blood, the second injection was the same. In the cat (weight 2·6 kilos) the first dose was 1·5 c.c. caffeine solution in 7·5 c.c. of blood, the second dose was 2·2 c.c. of caffeine in 9 c.c. of blood. We have found it advantageous

to dilute the caffeine solution with defibrinated blood rather than with saline solution.

At this point something may be said about the theory of caffeine diuresis. Up to the present time two theories have been put forward to explain it, (1) that the caffeine acts as a specific stimulant to the kidney cells[16] and (2) that it acts by causing vaso-dilatation[16], accompanied to some extent with a paralysis of the hypothetical reabsorptive mechanism of the tubules[17].

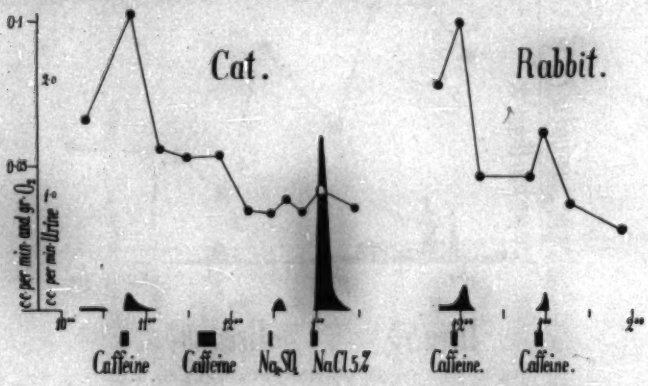

Fig. 7.

Fig. 8.

Oxygen and urine plotted as in Fig. 1.

We may first clear the ground to some extent by considering the action of caffeine on the vessels. In experiments to be subsequently described we have injected caffeine directly into the kidney vessels for the purpose of depressing the renal epithelial cells; the incidental effect noticed on the kidney vessels has been constriction. The following table shows the rate of flow through the kidney before and after the injection together with mean arterial pressure.

Rate of flow seconds per c.c. of blood		Mean arterial pressure	
Before	After	Before	After
0·8	1·8	78 mm.	72 mm.
0·9	1·2	90 mm.	103 mm.
1·9	3·9	138 mm.	122 mm.

The mechanism of the constriction is not clear, but presumably it is to some extent of central origin. Certainly in the first and third instances there was evidence, from the R.-P. tracing, of the caffeine having reached the general circulation to some extent: it is not the mechanism but the fact with which we are concerned. We do not wish to push these data beyond their legitimate sphere. It might be argued, and with some truth, that if there was not dilatation there was also no diuresis. Our point just here is merely this, that there was no gross inhibition of the tone of the kidney vessels, such as would be produced by pilocarpine on the heart or if injected into the artery of the salivary gland.

According to the first of the two views given above we should expect increased oxygen consumption on the part of the kidney cells, on the second view we should get decreased oxidation, an approximation to plasma in the constituents of the urine and large vasodilatation.

In Exp. 10, in which the caffeine was not injected into the renal vessels but into the general circulation, there was diuresis accompanied by only a very trivial increase in the blood flow, before and after the diuresis the blood flow was ·9 c.c. per second and during the diuresis 1·1 c.c. Similarly in Exp. 11, the rate of flow was increased from ·3 c.c. per second to ·4 c.c. This slight increase seems to us quite insufficient to account for the diuresis.

Nor was there a cessation of the metabolism of the cells suggesting diminished reabsorption of water, on the other hand there was increased metabolism.

The simple explanation then of caffeine diuresis is that caffeine stimulates the cell, and like so many protoplasmic stimulants ultimately poisons it. This view at once follows from the changes observed in the metabolism, and explains the reason why the cell should eliminate the drug. Moreover it is suggestive that in the caffeine diuresis observed in the rabbit, the chlorides in the urine were low 0·3—0·4 %. They presented no approximation to those of the plasma. In the percentage of chlorides therefore the urine resulting from caffeine contrasts markedly from the mechanical flow produced by Ringer's solution.

The poisoning action of caffeine upon the tubules accounts for the fact noticed by Loewi and known clinically that a second injection of caffeine gives rise to a smaller effect than the first.

Discussion of mechanical factors in diuresis.

It is desirable to make some analysis of the possible factors which might combine to render a mechanical flow of urine possible. Of these the most obvious is increased arterial pressure.

I. *Arterial pressure.* In our experiments this factor has not been the subject of special investigation so that it is quite unnecessary to publish a complete record of the arterial pressures which we have observed. We have taken a tracing in each experiment and we are convinced that other things being equal an increased arterial pressure tends to augment the flow of urine, but we have observed very many cases in which a copious diuresis has taken place without any such rise; we need only note that of Exp. 7 in which the flow of urine was the largest that we have obtained namely 5 c.c. per minute, nevertheless there was scarcely any change in the arterial pressure as compared with the previous period of rest. In the pre-diuretic period it was 126 mm., in the diuretic period 122 mm.

Indeed nothing could be more striking than the low pressure at which considerable quantities of urine may be secreted. In one case, see Fig. 9, for instance with an arterial pressure of 13 mm. of mercury, there was a secretion of 0·8 c.c. of urine per minute, the same animal secreted urine with an arterial pressure of 6 mm., maintained by massaging the thorax, after the heart had ceased to beat. It was one in which the blood had largely been replaced by a suspension of red corpuscles in Ringer's solution in a manner subsequently to be described.

II. *Venous pressure.* We are in agreement with de Souza[29] in so far as we are unable to regard increased venous pressure as the cause of diuresis. In some cases, after the injection of considerable quantities of saline solution, we have clearly had a considerable venous pressure. On the whole, other things being equal, a high venous pressure seems to go hand in hand with a weak heart; in cases where we measured the venous pressure during diuresis in vigorous animals it was very low, for instance at the height of the "normal saline" diuresis shown in Fig. 2, the venous pressure was less than 1 cm. of blood.

III. *Rate of flow.* In the majority of cases in which we obtained urine as the result of filtration there was an increase in the rate of flow of blood through the kidney. Such a condition with a constant arterial pressure would indicate an increased capillary pressure. We

have not however obtained increased rate of flow in all our experiments. The following are examples of cases in which the diuresis was not accompanied by any considerable increase of rate of blood flow.

Exp.:—	12	13	19	14	6	15	16	5
Rate before diuresis (secs. per c.c.)	1·2	1·2	1·8	0·9	2·5	·7	2·9	4·9
„ during „ „ „	1·2	1·1	2·0	1·0	2·5	·8	4·3	4·5

IV. *Dilution of the blood* has been the one constant change in the vascular conditions which has been present in all our experiments which have yielded diuresis without increased oxidation. We must therefore consider whether this in itself would tend to promote a flow of urine. Starling[19] has drawn attention to the importance of the osmotic pressure of the proteins in relation to the glomerular function. He accounts for the fact that a considerable capillary pressure is necessary for the production of urine in the glomerulus by supposing that the filtration can only take place when the capillary pressure exceeds the osmotic pressure of the non-diffusible constituents of the plasma. On the basis of this hypothesis it is clear that the injection of Ringer's solution, or water, or hypertonic salt solutions (which would attract much water from the tissues) would dilute the proteins in the plasma and would render the filtration more easy.

The possibilities here presented are very great. Suppose for instance that under ordinary circumstances the capillary pressure were just higher than the osmotic pressure of the proteins, place the former at 27 mm. and the latter at 25, the available pressure for filtration would be 2 mm. Halve the osmotic pressure of the proteins, *i.e.* make it 12·5, the available filtering pressure becomes 14·5 mm., a sevenfold increase. In Fig. 9 it appears urine could just be filtered under the conditions of the experiment at 6 mm. If this figure represented the osmotic pressure of the proteins there would have been on the above assumption a tenfold increase of nett filtering pressure.

It occurred to us that if the dilution of the blood were really an important factor, it should be possible by bleeding the animal to obtain a copious diuresis with much smaller injections of Ringer's solution than those used by Magnus, Frey etc., which were measured in litres. Before the injection of Ringer's solution the animal was therefore bled to the maximum extent consistent with life, oxygen being administered when necessary.

The following is the record of one such experiment.

Cat 1·75 kilos.
12.11. 10 c.c. Ringer's solution injected.
12.14. 17 c.c. of blood withdrawn and 50 c.c. of Ringer injected.
12.30. 25 c.c. of Ringer's solution injected.
12.34. 20 c.c. of blood withdrawn and 50 c.c. Ringer injected.
12.49. 50 c.c. of Ringer's solution injected.
12.56. 25 ,, ,, ,,
1. 1. 12 ,, ,, ,,
1. 7. 25 c.c. blood withdrawn and 50 c.c. Ringer injected.

During this time 272 c.c. of Ringer's solution were injected whilst 62 c.c. of blood were withdrawn, leaving a balance of about 200 c.c. or $\frac{1}{8}$ of the animal's weight if this 26 c.c. were secreted.

In such circumstances we were much struck with the low arterial pressure at which the urine might be secreted. We here give the portion of the tracing which represents the termination of this experiment.

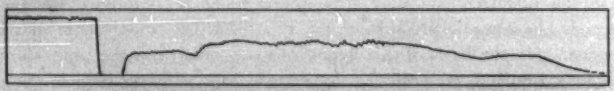

Fig. 9. $\times\frac{3}{4}$. Coincident with the first portion of the tracing 0·8 c.c. of urine per min. was flowing. In the second part the B.-P. was kept up mechanically and urine was secreted, none being secreted when there was no B.-P.

The urine and the plasma were analysed in this experiment. The analysis showed:

	Chlorides as NaCl	Urea	Albumen
Plasma	0·88 %	0·04 %	Absent
Urine	0·88 %	0·05 %	Absent

Everything in the above experiment, the comparatively small amount of the injection, the low blood-pressure, and the composition of the urine, pointed to our original assumption being justified. This experiment is only one of a number. That the urine did not appear as the result of gross mechanical injury is sufficiently attested by the fact that it contained no albumen (as shown by Heller's test) at any stage of the experiment, and also by the fact that in a similar experiment we replaced the original blood (which had been prevented from clotting by hirudin) in the animal; the diuresis after a time passed off and the urine secreted contained only 0·4 % of chloride.

We have endeavoured to eliminate factors other than dilution to a much greater extent than they were eliminated in the preceding experiment. With the object of maintaining the vascular conditions, *i.e.* the blood-pressure and the rate of flow, as constant as possible whilst at the same time diluting the plasma-proteins we conceived it possible to replace a considerable portion of the blood with a suspension of red corpuscles in Ringer's solution. For this purpose an animal was bled, the corpuscles centrifugalised out and washed, the process repeated and the corpuscles finally made up to about the original bulk

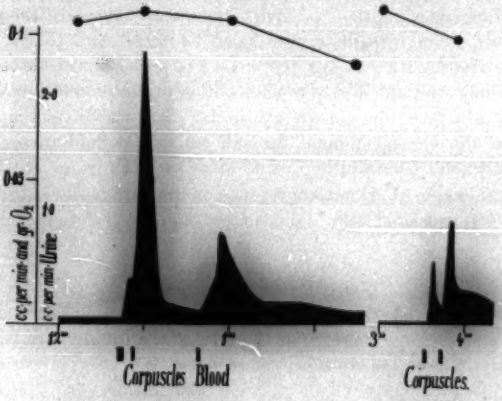

Fig. 10. Rabbit.

Fig. 11. Rabbit.

Oxygen and urine plotted as in Fig. 1.

of the blood. The record of such an experiment is given in Fig. 10. The animal which was the subject of the experiment was secreting 0·05 c.c. of urine per minute, its blood-pressure was 95 mm. of mercury; 22 cubic centimetres of blood was taken out and 25 c.c. of Ringer's solution was injected, the secretion at once rose to 0·4 c.c. per minute (see the notch on the record of the diuresis at 12.23), the arterial pressure was then but 52 mm. The suspension of corpuscles (25 c.c.) was put into the jugular vein, the arterial pressure at once rose to 84 mm., and the diuresis reached the very large figure (for a rabbit) of 2·35 c.c. per minute. The rate of flow of blood through the kidneys was slightly slower in the diuretic period than before it, being 1 c.c. in 4·3 seconds

as opposed to 1 c.c. 2·9 secs. The urine attained a value of 0·95 % of chlorides during the diuresis, the oxygen taken in presented scarcely any variation.

	Before diuresis	During		After
Oxygen taken in per grm. of kidney per minute	0·104 c.c.	0·108	0·105	0·09

Similar results were obtained in a second experiment of the same nature (Fig. 11). In it the oxygen taken in before the diuresis was 0·11 c.c. per gram per minute, during the flow 0·10.

V. *Other factors.* Whilst we are of opinion that the urine in these cases finds its way through the glomerulus by a process of filtration, we are conscious that we have by no means exhausted the physical factors which may regulate the degree of filtration. In the case of chloride-diuresis for instance, the change in saline concentration in the plasma may alter the degree of aggregation of the proteins and so alter their osmotic pressure; moreover a similar effect may, in fact must, take place in the cell membranes through which the fluid passes and so must alter their permeability, but whether such factors are considerable or inconsiderable we do not know; such as they are however they affect a much less restricted field in physiology than the secretion of urine.

Isotonic urine.

In the cases which we have just quoted, the urine secreted differs from the serum only in so far as the cells of the tubule have been able to modify it as it hurries down these passages. If we are correct in viewing it as being a modified glomerular filtrate, it is clear that if we can suspend the activity of the tubule cells, we should get a fluid in which the salts were jointly and severally isotonic with those of the plasma. In this we have appeared successful so far as the matter can be tested. The difficulty which such testing presents is that of ascertaining the extent to which some of the saline constituents are attached to the proteins of the plasma. This point is forming the subject of special investigation.

Here we may put down three methods for suspending the activity of the kidney cells, with each of which we have met with some degree of success: (1) poisoning with corrosive sublimate, (2) anæmia, (3) poisoning with caffeine. In the case of the first and third method, the problem is to poison the cells sufficiently for the purpose in hand, and at the same time to preserve the vascular conditions in a satisfactory state.

Our method of poisoning (suggested to us by Prof. Langley) has been to put a cannula in the superior mesenteric artery, to clamp the aorta in the vicinity of the cœliac axis, the vena cava is also clamped above the renal veins. The drug is injected through the arterial cannula, is kept in the kidney for the time required and is washed out with Ringer's solution, finding its exit through the cannula in the distal end of the vena cava. In this way a minimal quantity of the drug gets into the general circulation.

Before using such a method some enquiry must be made into the effect upon the kidney of cutting it off from the circulation. From the statements which have been made on the subject we were inclined to think that clamping would in itself probably kill the cells. We have made no experiments in which the kidney vessels have been clamped when they contained blood and therefore our observations are not quite

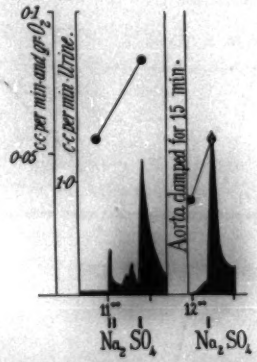

Fig. 12. Cat. Oxygen and urine plotted as in Fig. 1.

comparable with those of previous observers, but it is clear that a kidney survives the operation of clamping the vessels when the blood has been thoroughly washed out with Ringer's solution. This point is illustrated in Fig. 12. Sodium sulphate was used before and after the clamping (for 15 minutes) as a stimulus to the cells. The cells responded, as is shown both by the diuresis and the increased oxidation. Also the urine in each case was a typical secretion, at height of each diuresis it contained less than 0·05% NaCl. The general metabolism

of the kidney was not so great as before clamping, but its power to secrete remained.

1. *Sublimate poisoning.* The first poison which we employed was corrosive sublimate. It is known to constrict the vessels, indeed in the dose which we first used (2 c.c. of ·25 %), it did this with such effect that the blood flow was reduced from over 1 c.c. per second to 1 c.c. in 74 seconds. This experiment (Exp. 20) is worth putting on record partly on account of the fact that the venous blood which emerged even at this slow rate was bright red, the metabolism of the kidney having been for the time almost abolished; the gland which had been using 0·12 c.c. per gram, used only ·0011 c.c. after the sublimate. This experiment seems to dispose of the contention of those who hold that corrosive sublimate poisoning is due to anæmia produced by the vaso-constriction. Had this theory been correct the venous blood would

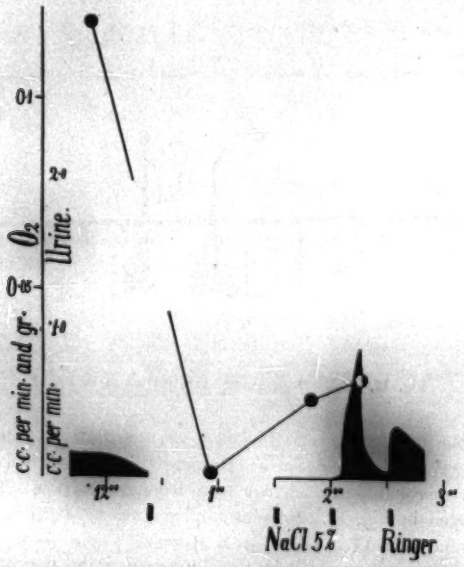

Fig. 13. Cat. Oxygen and urine plotted as in Fig. 1. The break in the O_2 line corresponds to the time during which the vessels were clamped, the signal underneath it to the injection of sublimate.

have been completely reduced. Clearly the sublimate acts on the kidney cell.

Usually the kidney in the cat or rabbit at the beginning of an experiment is using from 0·07 to 0·1 c.c. of oxygen per minute. Experience has shown us that this must be reduced to something like 0·02—0·03 c.c. before we can claim to have abolished the active influence of the tubule cells. At this point we may give details of Exp. 21 in which a rapid diuresis unaccompanied by increased oxidation was produced by injection of sodium chloride; the kidney at the same time had been poisoned with sublimate till its metabolism had fallen to 0·013 c.c. of oxygen per gram per min.

	Rate of blood flow	Do. of urine	Oxygen used up	Chloride in urine
Before sublimate	1 c.c. in 2·5″	—	0·06 c.c.	—
After ,,	1 c.c. in 33″	none	0·013 c.c.	—
During chloride diuresis	1 c.c. in 24″	rapid	0·014 c.c.	·8 %

In the case of Exp. 20, Fig. 13, it is of interest to observe that with the tubules poisoned, it was also possible to evoke a considerable diuresis with 5 % NaCl as well as with Ringer's fluid, the final urine contained 0·8 % NaCl and the O_2 consumed was but 0·026 c.c. per gram per minute.

2. *Anæmia.* The second series of experiments in which we have obtained what appeared to be isotonic urine was that already alluded to in which we administered considerable quantities of Ringer's solution after profuse bleeding. In some such cases the tubules appear to have been temporarily asphyxiated, as the blood reaching them contained an inadequate supply of hæmoglobin.

The following data were derived from two such experiments (Exps. 18 and 3).

Exp. 18.

Period	Urine	NaCl	Urea	Oxygen taken up
I.	After 50 c.c. Ringer	—	—	0·05 c.c.
II.	After bleeding and Ringer ...	0·67 %	0·22 %	0·06 c.c.
III.	After further bleeding and injection	0·83 %	0·04 %	0·017 c.c.
IV.	Further injections	0·88 %	0·05 %	0·018 c.c.
	Blood at end of exp.	0·88 %	0·04 %	...

It will be seen that the very low oxygen consumption of the last two periods was associated with urine which must have been a very close approximation to serum as regards crystalline bodies. Probably the blood, or rather the circulating fluid, containing as it did but a small fraction of the original hæmoglobin, was completely reduced.

In Exp. 3 (of the same character) the chloride in the urine rose from ·37 % before the bleedings to ·83 %, the oxygen taken in at the final stages of the experiment was ·033, ·030 and ·026 c.c. per gram of kidney per minute. In the last analysis it corresponded to about ⅛ of what the arterial blood at the commencement of the operation might have yielded if the kidney had taken all its oxygen, and as it is scarcely possible that the blood at the end can have been more than ¼ of the concentration that it was at the beginning, it is probable that the cells were not able to get enough to give them the opportunity of doing work.

3. *Caffeine poisoning.* A single experiment in which the kidney was poisoned with caffeine, gave a similar result, viz. that when the metabolism of the kidney had fallen to something approaching ⅓—¼ of its former value isotonic urine was secreted.

The following data may be given.

Exp. 15.	Urine per min.	NaCl %	Urea %	Albumen	Oxygen used
Normal kidney ...	·2 c.c.	0·85	1·03	none	0·13
Diuresis after caffeine poisoning ...	1 c.c.	0·95	·07	trace	0·04
Blood corresponding to diuresis ...	—	0·9	·065	—	—

In this case 10 c.c. of a 5 % solution of caffeine sodium benzoate had been injected into the superior mesenteric artery as above described and the kidney then clamped for five minutes. The immediate cause of the diuresis was the injection of 125 c.c. Ringer's fluid. That the kidney was not asphyxiated in this case is shown by the fact that the venous blood emerging from it was about 50 % saturated with oxygen.

The question of reabsorption.

In the foregoing section we have spoken of the glomerular filtrate being modified in its passage down the tubule. Whilst the major theme of the present paper is a proof that filtration can exist, it is scarcely possible to leave the subject without some enquiry into the much vexed question of whether the modification of the urine by the tubule is a process of secretion or reabsorption. So far as we can contribute anything to this controversy it is the data which are available in the case of kidneys whose tubules have been poisoned. Of these we would say:

That the poisoning of the tubule seems to restrict the amount of urine which can be obtained as the result of sodium sulphate diuresis; and to deprive it of its typical character. Such urine under normal

circumstances is very poor in chlorides (they may even be absent) and very rich in sulphates. On the reabsorption theory it is arrived at by a process of complete or almost complete reabsorption of chlorides and reabsorption in a very great measure of water. Were these reabsorptive functions done away with, a urine would result which was enormously greater in volume than a normal sulphate diuresis and containing a large quantity of chlorides, upwards of 0·8 %, or perhaps more.

In the matter of bulk then the reabsorption theory demands precisely the opposite of what we have obtained. We quote two experiments in which the kidneys have been poisoned and have yielded but a scanty secretion with sodium sulphate. That there was nothing in the nature of the case to prevent a copious filtration is shown by the control experiments which were performed in each case with sodium chloride.

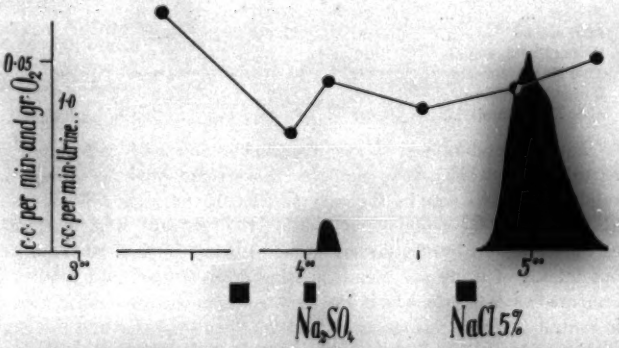

Fig. 14. Cat. Oxygen and urine plotted as in Fig. 1. The first signal represents the poisoning with caffeine.

These experiments (10 and 22) are figured in Figs. 7 and 14; the leading data are as follows.

	Exp. 10	Exp. 22	Control (1) unpoisoned	Control (2) unpoisoned
O_2 metabolism before poisoning with caffeine in c.c. per grm.	0·066	0·063	0·03	0·040
Do. after caffeine	0·034	0·031	—	—
Do. during Na_2SO_4 diuresis ...	0·038	0·045	0·184	0·089
Do. after Na_2SO_4 diuresis ...	0·034	0·037	0·036	0·048
Do. during NaCl diuresis ...	0·041	0·043	0·034 0·036	0·033 0·043
Do. after NaCl diuresis ...	0·035	0·050	0·029	0·033

The above-mentioned figures show:

(1) That the caffeine virtually abolished the specific effect of the sodium sulphate. The oxidation was much the same in Exps. 10 and 22 during the Na_2SO_4 diuresis as immediately before and immediately after it. In this control the oxidation was much increased during the Na_2SO_4 diuresis.

(2) Therefore such urine as came with the sulphate might be expected to be isotonic.

(3) This on the secretory theory should be small in amount, on the reabsorption theory great, unless the vascular conditions were modified in such a way as to interfere with the result, these therefore must be considered in the following table:

No. of c.c. of blood going through 1 gram. of kidney per sec.

	Exp. 10	Exp. 22		Control 2
Normal	1·9	1·1		2·5
After caffeine ...	1·0	0·6	—	—
During Na_2SO_4 diuresis	2·1	1·6	2·0	3·6
After Na_2SO_4 diuresis	0·9	0·5	1·2	2·5
During NaCl diuresis...	3·1	2·7	2·5	1·6
After NaCl diuresis ...	0·9	1·3	1·6	0·9

Exp. 10 and control 1 are remarkable for their consistency as regards rate of blood-flow, moreover the arterial pressure during the sulphate diuresis was in the case of Exp. 10 83 mm. and control 1 82 mm.; thus the vascular conditions in the two cases were as similar as can be shown by measurements, nevertheless in Exp. 10 there was but 0·1 c.c. of urine per minute passing down the poisoned tubules as compared with 0·5 c.c. which was secreted from the unpoisoned kidney in control 1. Now this last contained 2·3 % of sulphates and therefore on the reabsorption theory as it is unlikely that the plasma contained more than 1 % this would represent a glomerular filtrate of at least $0{\cdot}5 \times 2{\cdot}3 = 1{\cdot}15$ c.c. per minute. This then we might have expected to get under the same vascular conditions in Exp. 10, instead we get less than $\frac{1}{10}$ of that amount. It is however fair to say that the kidneys in the case of Exp. 10 were only about half the size of what they were in control 1; to make the comparison fair we should consider that we got 0·2 c.c. where we looked for 1·15.

These facts are again controlled by the fact, that in the subsequent diuresis which was brought about mechanically (NaCl in one case, Ringer in the other) there was a rough equality in each case, both in the vascular conditions, the oxygen used up and the urine secreted.

Mechanical diuresis.

(All measurements in c.c. per grm. of kidney per minute.)

	Oxygen	Blood	Urine
Exp. 10	0·041	3·1	·056
Control 1	0·036	2·5	·044

From the above it will be seen that the two experiments ran a very consistent course, the slight balance of urine being on the side of Exp. 10.

The following are the details of the urine:

	Exp. 10	Exp. 22		Control 1		
Period	Quantity of urine, c.c.	Quantity, c.c.	NaCl %	Quantity, c.c.	NaCl %	Na_2SO_4 %
Normal	0·03	0+	0·6	0·08	0·78	0·7
After caffeine ...	none	none	—	—	—	—
During Na_2SO_4 diuresis	0·1	0·2	0·6	0·5	0·17	2·27
After Na_2SO_4 diuresis	none	none	—	none	—	—
During NaCl diuresis	1·5	1·3	0·6	2·1	0·78	0·37
After NaCl diuresis ...	none	0·05	—	none	—	—

The reader should note:

(1) That from the poisoned kidney the percentage of chlorides during this sulphate diuresis is equal to that secreted during the chloride diuresis.

(2) In this control the chloride during this sulphate diuresis is meagre as compared with the above and with what is secreted before and after.

Therefore the caffeine has abolished this characteristic paucity of chlorides during sulphate diuresis.

Whilst the possibility of some slight degree of absorption cannot be categorically denied we consider that two factors only are established, filtration in the glomeruli and secretion in the tubules, and that it is possible with their aid to account for all the facts of which we have a knowledge.

Conclusions.

1. The diuretics which we have studied fall into two groups (1) those which produce urine without alteration in the gaseous exchange of the kidney (Ringer's solution and NaCl in hyper- and hypotonic solutions); (2) those which cause increased gaseous exchange (urea, caffeine, sodium sulphate), to these may be added phloridzin

(Barcroft and Brodie). In the case of urea and caffeine there is a definite poisoning action as shown by subsequent depression of the gaseous exchange.

2. The distinctive features of the urine produced by the second class are attained by a process of secretion on the part of the tubules, rather than by a process of reabsorption.

We have failed to find any degree of inhibition during diuresis, suggesting that there is no suspension of a reabsorptive function. In the case of kidneys with the tubules poisoned the evidence points rather to their having lost the power of secretion than to their having lost that of reabsorption.

3. After poisoning the cells of the tubules it is possible to get a flow of urine with the first class (and a restricted flow with sodium sulphate due to the merely mechanical properties of the salt) which appears to be isotonic with the serum.

4. Clamping the kidney for 15 minutes after washing out the vessels with Ringer's solution, does not abolish its power of secretion.

5. Amongst the mechanical factors which play a part in the production of urine as the result of injection of Ringer's solution, is the lowering of the osmotic pressure of the blood proteins due to dilution, hence after repeated bleedings and injections of Ringer's solution, urine can be filtered at a very low pressure.

(A portion of the expenses of the above research has been defrayed by a Grant from the Royal Society.)

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We do not of course profess to have given a complete bibliography of the subject; such will be found in the text-books of Nagel or of Oppenheimer.

ON THE GOVERNING INFLUENCE OF ENVIRONMENT ON ENZYMIC ACTION.

By F. W. PAVY, M.D., LL.D., F.R.S., with the collaboration of H. W. BYWATERS, D.Sc., Ph.D., F.I.C.

(From the Physiological Laboratory of the University of London, South Kensington.)

The recent advance of knowledge in connection with enzymic action has opened out ground that puts us into possession of a new field of inquiry in relation to metabolism. Intracellular enzymic action is now recognised as an important determining factor in connection with the chemical changes taking place in the organs and tissues of the body. The liver has been long regarded as an organ invested with special interest in relation to enzymic action. It is a matter of common knowledge that the polysaccharide—glycogen—present in it undergoes, under specific conditions, transformation into sugar, and it is open to demonstration that it is through enzymic action that the transformation is effected. All are familiar with the active production of sugar that takes place in the liver immediately after death and the contrast that exists in the state of the liver with regard to sugar content at the moment of death and a short time afterwards. It has been felt that the difference between the *ante-mortem* and the *post-mortem* states ought to be susceptible of being accounted for under the view that enzymic action is at the foundation of the matter.

The problem that presented itself for solution, created a demand for a knowledge of how enzymic action is affected by influencing agencies. If under one set of conditions a play of action is observable which under another set of conditions does not similarly occur, it may be concluded that some modifying influence must be at work to create the difference. Upon this line of reasoning, an experimental inquiry was started in the direction of investigating the governing conditions belonging to enzymic action.

Invertase.

It was thought that yeast was likely to afford the best medium to work with on account of its simple character and the readiness with which it is susceptible of being experimentally dealt with.

Invertase was selected as the enzyme to take, and its action was measured by the determination of the amount of invert sugar produced, from the saccharose used, by means of the ammoniated cupric (Pavy's) test. Seeing that saccharose with no cupric oxide reducing power is straightway transformed into a cupric oxide reducing body (invert sugar), which is with such ease and speediness susceptible of estimation, nothing could stand more desirably placed for what is wanted.

In the experiments that follow, the yeast employed consisted of the pressed yeast sold by bakers under the name of "Balloon Yeast," and care was taken to obtain it in a fresh state. An aqueous extract was in the first place made at room temperature. The yeast was well shaken with the water and allowed to remain in contact for a few hours and then filtered through paper. The product was found to possess a limited amount of enzymic power varying with different samples. For instance, taking 10 grms. of yeast and 100 c.c. of water and extracting for four hours, 5 c.c. of the product incubated for two hours at a temperature of 40° with 1 grm. of saccharose and water to make an incubating volume of 20 c.c., which represents the standard incubating volume employed, the amount of invert sugar produced has usually ranged from say 20 to 50 mgs., the figures, however, sometimes running up to 100 or more. In several instances where 2 c.c. quantities of extract have been employed, no measurable amount of invert sugar has been formed.

Two drops of a 60 per cent. caustic potash solution added to the contents of the flask at the termination of incubation arrests further enzymic action and permits of the invert sugar determination being made on the following day. Preparatory to titration, the incubation product, without being boiled, is brought to a given volume and, if necessary, thrown on to a dry filter. The presence of alkalinity, it must be understood, is in all cases to be secured before boiling with the ammoniated cupric test.

The sugar found may be put down as derived from saccharose transformation, seeing that the yeast at starting has been found to be practically free from cupric oxide reducing sugar.

Upon the strength of the results obtained by Harden and Young[1] showing the effect of boiled yeast juice in increasing the activity of zymase, the yeast extract to which reference has been made was treated with boiled yeast extract previous to being subjected to incubation with saccharose. The results yielded by this mode of experimenting were of a surprising nature, a conspicuously marked increase of enzymic action being observed to be brought about.

The subjoined table serves to show the character of the actual effect produced. The boiled yeast extract was made from 20 grms. of yeast boiled for 20 minutes in 100 c.c. of water. It possessed in itself no cupric oxide reducing power.

Unboiled aqueous extract of yeast incubated with saccharose in the presence of water and of boiled yeast extract.

Amount of unboiled extract used, c.c.	Invert sugar found after 2 hours' incubation at 40° with 1 grm. of saccharose and	
	With water, mgs.	With 5 c.c. of boiled yeast extract, mgs.
10	15·8	209·8
5	16·9	248·0
5	51·2	394·0
5	22·8	248·0
2	10·8	125·0
2	0	59·1
2	0	80·2
2	0	57·8
2	0	94·6

The figures in the middle column show that when the unboiled aqueous extract of yeast was incubated with saccharose without any added boiled extract, only a limited amount of invert sugar was produced, whilst greatly increased amounts were found when incubated in the presence of the added boiled extract. In the last four instances with 2 c.c. of the unboiled extract used, no indication of the presence of invert sugar was obtained in the absence of the boiled extract, whilst after incubation in association with the added boiled extract, 59, 80, 57 and 94 mgs. of invert sugar were met with.

In the following experiment, information was sought regarding the effect of extraction for different lengths of time. Extraction, for the lengths of time represented below, was made from 10 grms. of yeast to 100 c.c. of water. 5 c.c. filtered portions were incubated with 1 grm.

[1] This *Journal*, xxxii.

of saccharose in the presence of water on the one hand, and in that of 5 c.c. of boiled extract, prepared as usual from 20 grms. of yeast and 100 c.c. of water, on the other.

Effect of incubation with boiled yeast extract and with water.

Duration of extraction	Invert sugar found after 2 hours' incubation at 40°: With water, mgs.	With boiled yeast extract, mgs.
Extraction at room temp. (21°).		
Taken at once	0	52·0
Taken in 1 hour's time	3·2	57·4
,, 3 ,,	5·8	68·4
,, 5 ,,	8·1	90·7
,, 20 ,,	7·2	106·1
Extraction at incubation temp. (40°).		
Taken at once	0	52·0
Taken in 1 hour's time	5·8	84·8
,, 3 ,,	27·1	120·9
,, 5 ,,	43·3	216·7
,, 20 ,,	81·2	245·2

For the clear representation of the part played by the boiled yeast extract, a given quantity of the unboiled extract was incubated with saccharose and varying quantities of boiled extract. Tap water was used in connection with the experiment, and 5 c.c. was the amount of unboiled extract employed with 1 grm. of saccharose.

Unboiled yeast extract incubated with saccharose and varying quantities of boiled extract.

Amount of boiled yeast extract used, c.c.	Invert sugar found after 2 hours' incubation at 40°, mgs.
0	0
0·1	5·1
0·2	7·6
0·35	11·8
0·5	19·5
1·0	40·3
2·5	86·2
5·0	113·6

From the evidence thus furnished, it became manifest that a potency exists in the boiled extract, and the next procedure to adopt was to determine the precise agency to which the potency is attributable. Yeast was incinerated in a platinum capsule, and the incinerated product substituted for the boiled yeast extract employed in the former experiments. The results showed that the potency did not lie in the

inorganic portion of the yeast. In view of Harden and Young's[1] discovery of the activating influence of phosphates on zymase action, it was thought at first that invertase might be influenced in a similar way. The following experiments suffice to show that such is not the case.

Unboiled aqueous extract of yeast incubated with saccharose and incinerated yeast.

Amount of incinerated product employed	Invert sugar found after 2 hours' incubation at 40° of 1 grm. of saccharose and 2 c.c. of unboiled extract with incinerated yeast		
	Exp. 1 mgs.	Exp. 2 mgs.	Exp. 3 mgs.
None	71·0	53·2	54·2
From 1 gm. of yeast	15·8	52·1	44·6
,, 2 ,, ,,	9·1	19·9	7·9
,, 5 ,, ,,	4·2	12·5	3·6

The incinerated product was found to be neutral or slightly alkaline to litmus test paper.

Seeing that the potency of the boiled yeast extract must be looked for in another direction, attention was given to the acidity which was observed to belong in a marked manner to the extract. Unboiled yeast extract was incubated with saccharose in the presence of acetic acid, which, in view of the collateral circumstances existing, was considered to be the best to employ in place of the boiled yeast extract, and it was at once observed that the phenomena occurring ran on perfectly parallel lines.

In the two following experiments, unboiled aqueous yeast extract was incubated with water, with boiled yeast extract, and with acetic acid.

Incubation with water, with boiled yeast extract, and with acetic acid.

Amount of unboiled aqueous extract used, c.c.	Invert sugar found after 2 hours' incubation at 40° with 1 grm. of saccharose and		
	With water, mgs.	With 5 c.c. boiled yeast extract, mgs.	With 1 c.c. $\frac{N}{10}$ acetic acid, mgs.
5	6·9	129·3	119·0
5	67·6	438·6	500·0

Thus 1 c.c. of $\frac{N}{10}$ acetic acid stood equivalent in its effect to 5 c.c. of boiled yeast extract, and the relative acidity of the two was found by observation to closely correspond.

From what has been said, it follows that the same kind of result is obtainable no matter whether boiled yeast extract or acetic acid is employed. In the subjoined experiments of a parallel nature to those adduced in connection with boiled yeast extract, acetic acid was substituted for it.

[1] *Proc. Roy. Soc.* 77 B. 1906.

Unboiled yeast extract incubated with saccharose in the presence of water and of acetic acid.

Amount of unboiled yeast extract, c.c.	Invert sugar found after 2 hours' incubation at 40° with 1 grm. of saccharose and	
	With water, mgs.	With 1 c.c. $\frac{N}{10}$ acetic acid, mgs.
5	75·8	304·9
5	67·6	500·0
5	25·0	238·1
5	108·7	500·0

From the details in this table, the activating effect of acetic acid is seen to be quite equal to that of boiled yeast extract.

Activating effect of varying quantities of acetic acid on an incubating medium containing 0·05 % of yeast (not extract) and 1 grm. of saccharose.

c.c. of $\frac{N}{10}$ acetic acid present	Invert sugar found after 2 hours' incubation at 40°, mgs.
·1	6·6
·3	50·0
·5	182·9
·7	277·8
1·0	267·6
3·0	270·2
10·0	270·2

The effect of the acid is seen to be an increasing one until a certain quantity is reached, when, within the limits here employed, it remained stationary. The following experiment, however, shows that when carried beyond a certain point, the acid exerts a diminishing effect.

Succession of results from acetic acid carried up to and beyond the optimum point.

Amount of acetic acid present	Invert sugar found after 2 hours' incubation at 40° with 1 grm. of saccharose and 5 c.c. of unboiled yeast extract, mgs.
0	108·7
0·5 c.c. $\frac{N}{10}$	500·0
1 ,,	500·0
2 ,,	530·8
5 ,,	506·8
0·5 c.c. $\frac{2N}{1}$ = 10 c.c. $\frac{N}{10}$	506·8
1 ,, = 20 ,,	360·6
5 ,, = 100 ,,	72·5

In the absence of acetic acid, the incubated saccharose and unboiled yeast extract gave 108·7 mgs. of invert sugar. With 5 c.c. of $\frac{N}{10}$ acetic acid, an instantaneous rise to 500 mgs., it is seen, occurred, and this amount remained practically constant till the equivalent of 20 c.c. of the $\frac{N}{10}$ acid was reached, when it fell to 360·6, and with 100 c.c. to 72·5.

In connection with the results that have been given, evidence is adducible to show that the effect observed is not attributable to an action *per se* of the boiled yeast extract and the acetic acid. Incubation of saccharose with these agents alone has not been found to be attended with the production of any material amount of invert sugar.

From what has preceded it has been seen that an aqueous extract obtained from fresh yeast possesses a variable and comparatively insignificant amount of invertase enzymic power and that this becomes greatly increased in the presence of boiled yeast extract, and of acetic acid. A marked change of condition is produced by bringing yeast into a dry state, by grinding it up with kieselguhr, and by treatment with alcohol or with acetone. The effect is to give to the product the property of yielding to an aqueous extract a greatly augmented enzymic power and to diminish or remove the activating faculty of boiled yeast extract, and of acetic acid. It seems as though the potential thermo-labile portion of the enzyme became activated in the treatment and thus acquired a greater or less approach to the possession of full enzymic power. In some instances comparatively high initial figures for generated invert sugar have only been to a moderate extent raised by incubation with the boiled yeast extract, and in some not at all, showing that no capacity was left for further activation.

Effect of carbonic acid. Carbon dioxide produces the same activating effect upon the dynamic basis of invertase as acetic acid. The fact of its acting like acids generally in promoting enzymic action has not escaped the observation of others. For the sake of ascertaining the position it occupies as an activator, its power has been measured by comparing it with that of acetic acid and it is seen from the subjoined experimental results that the two stand about on an equality.

It is not to be expected that the passage of air instead of carbon dioxide through the incubating admixture would be attended with any show of effect, and such has been found to be the case. In one case, where an extract from yeast that had been ground up with kieselguhr was employed, identically the same figures for invert sugar—367·6—were obtained, when incubated with water, with and without the passage of

air through the incubating medium. In another case, the figures without the passage of air were 726·7, and with, 765·3.

Form in which yeast was employed	Invert sugar found after 2 hours' incubation at 40° of 1 grm. of saccharose with		
	Water, mgs.	Passage of CO_2, mgs.	1 c.c. $\frac{N}{10}$ acetic acid, mgs.
Extract	Under 5	271·5	297·5
Extract	6·5	357·3	462·8
2 % aqueous admixture	130·1	202·7	210·5
2 % ,, ,, ...	24·5	175·5	185·0
2 % ,, ,, of dried yeast	290·5	735·0	757·5
2 % ,, ,, ,, ,,	41·7	707·6	646·6

Effect of treatment with basic substances.

The tenor of the evidence supplied by the foregoing inquiry into the position of acids in relation to invertase enzymic action is supported by the effect of basic substances on the process. The broad fact has long been known that alkalies exert an inhibiting influence over the process.

Experiments have been undertaken with various basic substances and, as the subjoined narration of results shows, the dynamic portion of the enzyme is rendered adynamic in their presence. Employed in small amount, this is all that occurs, and under the removal of the basic character of the medium by the addition of an acid, the lost power becomes restored. This only applies in the presence of limited amounts of basic substance. With larger amounts, the restoration of power fails to be capable of being brought about. The potential part of the enzyme is in fact killed.

Ammonia.

	Invert sugar after 2 hours' incubation at 40° with 1 grm. of saccharose in an incubating volume of 20 c.c., mgs.
5 c.c. aqueous extract of yeast (slightly acid reaction)*	862·1
Ditto, treated with 0·25 c.c. $\frac{N}{10} \cdot NH_3$	168·9
Ditto, ,, 0·5 ,, ...	46·3
Ditto, ,, 1·0 ,, ...	14·9
A duplicate set of experiments was conducted, and after the first incubation the products were acidified with acetic acid and incubated again, when the following results were obtained:	
Product that had been treated with 0·25 c.c. $\frac{N}{10} \cdot NH_3$	806·5
,, ,, ,, 0·5 ,,	416·7
,, ,, ,, 1·0 ,,	14·5

* Whenever not otherwise stated, litmus paper has been used as the indicator.

It is seen that after acidification of the product containing the 0·25 c.c. of ammonia, the invert sugar subsequently found nearly equalled that arising from the initial extract that had not been treated with ammonia. From this it may be inferred that the dynamic portion of the enzyme had not been essentially damaged by the amount of ammonia used. Where 0·5 c.c., however, was used, the figures for the invert sugar were reduced to about half, showing that considerable damage occurred, and where 1 c.c. was employed, it may be considered that practically full destruction was induced.

Sodium carbonate. An aqueous extract of yeast, presenting a slightly acid reaction and giving, after incubation with saccharose, 520 mgs. of invert sugar, on being brought to a neutral state with 1 per cent. solution of sodium carbonate previous to incubation with saccharose, gave 15·5 mgs. of invert sugar. Deprived of acidity, enzymic action was practically annulled. With acidity restored by the addition of acetic acid to another neutralised portion, the invert sugar found after incubation amounted to 672·4 mgs.

An aqueous extract of yeast with an acid reaction and giving 446·4 mgs. of invert sugar on incubation with saccharose, after being made just alkaline with a solution of sodium carbonate and then incubated, gave 6·8 as the figures for invert sugar. Another portion similarly made just alkaline and incubated and then acidified and incubated again gave the figures 9·1, a result which showed that the sodium carbonate that was present during the first incubation sufficed to kill the dynamic portion of the enzyme.

Lime-water. Adopting the same line of experimenting with lime-water, an extract of yeast which had been ground up with sand and gave incubation figures of 446·4, was treated with 0·5 c.c. lime-water, by which the incubation figures were reduced to 97·6, and with 1 c.c. of lime-water, by which they were brought to 18·4. Corresponding portions after acidification and incubation again, gave in the case of the 0·5 c.c. of lime-water, 551·5, and in that of the 1·0 c.c., 475·3. The lime-water had thus only checked activity without destroying the dynamic portion of the enzyme.

Calcium carbonate. An extract of yeast which had been ground up with kieselguhr and in which after incubation 1013·5 mgs. of invert sugar were found, gave after being shaken with calcium carbonate, filtered, and incubated, an indication of the presence of 806·5 mgs. An acidified portion of the filtered liquid showed after incubation the presence of 1071·4 mgs. A portion of the extract incubated in contact with calcium carbonate showed the presence of 872·1 mgs.

Other experiments have been conducted with magnesium oxide, magnesium carbonate and barium carbonate, each of which has been found to be more effective than the calcium carbonate in restraining enzyme activity, but the line of action has stood the same.

Tap-water and distilled water. The basic material in tap-water is even sufficient to produce a decided influence on the enzymic activity of invertase. In the following set of experiments, yeast was added to distilled water and tap-water and incubation with saccharose in the usual manner carried out. The amount of yeast present in the 20 c.c. incubating liquids was 0·05 per cent.

Invert sugar found in	
Distilled water product, mgs.	Tap-water product, mgs.
260·3	11·4
309·4	35·1
130·1	55·8
135·8	0
175·4	0
185·2	0

In other experiments, extracts prepared on the one hand with distilled water and on the other with tap-water were used. In one, whilst the amount of invert sugar found in association with distilled water was 352·0 mgs., it was 15·1 in association with tap-water. In another, the respective amounts were 140·8 and 55·6; and again in another 89·2 and 14·5.

These experiments strikingly illustrate the part played by environment in governing enzymic action.

DIASTASE.

The knowledge gained through the medium of invertase was taken as a basis for studying the mechanism of the action of diastase, the enzyme concerned in the hydrolysis of starch and its congener glycogen, which, belonging to the animal system, brings us into touch with the chemistry of animal life.

Estimation of diastatic action. The enzymic action occurring in connection with the matter under consideration consists of transformation of glycogen into sugar. There are, therefore, the two factors—loss of glycogen and genesis of sugar—presented for being dealt with in measuring the amount of action ensuing.

The first is a very lengthy process, and, from what occurs with the use of an alkali[1] as a solvent of the material operated upon, can scarcely be considered to reliably give the information wanted. The initial stage of transformation is into a dextrin approximating closely to the glycogen started with, and it happens that this product, which constitutes a result of very slight enzymic change, is, unlike glycogen, readily susceptible of destruction on being boiled with potash, a circumstance which leads to its disappearance in the process adopted, and hence to its being reckoned as constituting the result of as much action as where the change has been carried up to its terminal point.

The estimation of diastatic action through the cupric oxide reducing power of the product is a procedure which admits of being easily and speedily carried out, and at the same time may be considered to give the kind of information that is wanted. As in the case of invertase, it constitutes the process that has been employed in the investigation that is about to be detailed. Some have suggested that what is referred to under the head of glycolysis may exert a detracting influence on its value, but there is no valid ground to support the idea.

Oyster Diastase.

The oyster was to begin with made use of as a medium of investigation on account of its special suitability for the purpose. Consisting, as the central portion of it does, mainly of liver and store of glycogen, it offers just the character of material that is wanted for subjection to experiment. The portion referred to was dissected away from the rest and reduced to a finely divided state in a mortar.

In the living oyster, the liver, as in living animal structures generally, contains a certain small amount of sugar. This increases after the death of the animal. The cupric oxide reducing power of the sugar is low compared with that met with in connection with the liver of the warm-blooded animal. It is ordinarily below that of maltose.

In the experiments to be detailed, it will be noticeable that the same order of result is traceable in the case of the diastase of the oyster as in the case of invertase. The extent of action, however, it will be perceived, is much more limited. On account of the less extent of action occurring, the incubation at 40° was carried on for four hours instead of for two hours, the time given for invertase. On removal from the incubating chamber, the product was boiled to check further action,

[1] Vide Pavy, *Physiology of the Carbohydrates*, pp. 151–2. 1894.

and after being brought to a given volume, was made slightly alkaline, and then thrown on to a dry filter and titrated for sugar with the ammoniated cupric (Pavy) solution.

The subjoined tabular representation refers to experiments in which the effect of boiled oyster extract and of acetic acid was ascertained. The boiled extract was found to give with litmus a certain amount of acid reaction. The sugar present in the fresh oyster product and in the boiled extract was determined and deducted from the figures obtained after incubation, so that the figures given represent the actual production of sugar occurring. The nature of the sugar produced not being shown by the process, the cupric oxide reduction revealed is expressed as representative of glucose.

Unboiled oyster extract incubated with boiled oyster extract and with acetic acid.

		Sugar expressed as glucose found after 4 hours' incubation at 40° with			
				$\frac{N}{10}$ acetic acid	
	Incubating volume, c.c.	Water, mgs.	Boiled oyster extract, mgs.	c.c. added	mgs.
Oyster pounded up with water	20	112·4	136·0	—	—
Oyster ground with kieselguhr and divided into portions equivalent to 5 grms. of oyster	20	170·3	271·4	3	284·5
				1	225·3
Oyster ground with sand and water and squeezed through linen	25	33·7	—	3	129·9
Ditto, and 0·250 grm. of soluble starch added	40	60·2	—	3	190·4
Ditto Ditto ...	40	49·3	—	3	165·1
Ditto Ditto ...	40	51·8	67·8	10	128·8
Ditto Ditto ...	40	67·3	88·0	10	180·2

Unboiled oyster extract incubated with varying amounts of acetic acid.

	Sugar expressed as glucose found after 4 hours' incubation at 40° with the addition of 0·250 grm. of soluble starch. Incubation volume 20 c.c.	
Amount of acetic acid present	Exp. 1, mgs.	Exp. 2, mgs.
0	60·2	49·3
1 c.c. $\frac{N}{10}$	117·2	—
3 ,,	190·4	165·1
10 ,,	126·9	124·3
1·5 c.c. $\frac{2N}{1}$ $\left(=30 \text{ c.c. } \frac{N}{10}\right)$	99·3	—

Under an increasing strength of acid up to a certain point the amount of sugar produced is seen to rise, and then, under a further addition, to fall.

Effect of carbonic acid. In the case of invertase the effect of carbon dioxide was seen to stand pretty closely approximate to that of acetic acid in promoting enzymic action. Oyster diastase has also been made the subject of experiment, and the results obtained show the same line of action although upon a greatly reduced scale.

25 grms. of the oyster substance were pounded with sand and water, and the extract obtained by squeezing through linen was made up to 50 c.c. 10 c.c. portions were worked with in an incubation volume of 20 c.c. The 10 c.c. control boiled at once contained 9·5 mgs. of sugar and this was subtracted in obtaining the subjoined figures expressive of the results yielded.

	Sugar expressed as glucose found after 4 hours' incubation at 40°, mgs.
Incubated with water	83·1
,, ,, 3 c.c. $\frac{N}{10}$ acetic acid ..	91·8
,, ,, transmission of carbonic acid	106·7

Nature of the carbohydrate present in the undermentioned conditions. The following experiment shows that the effect of the acid is not only to increase the gross amount of sugar but to give a higher cupric oxide reducing character to the sugar produced. The product was treated with alcohol to dissolve out the sugar and get rid of the other carbohydrate material present. The sugar obtained was then hydrolysed in the usual manner.

	Oyster extract	
	Before hydrolysis, mgs.	After hydrolysis, mgs.
Boiled at once	36·8	89·2 = K 41
Incubated with water	67·6	121·9 = K 55
,, ,, 3 c.c. $\frac{N}{10}$ acetic acid	156·2	181·8 = K 86

A point to be noted in these results is that, with incubation in the presence of acetic acid, a stage of hydrolysis considerably above that of maltose was reached.

Influence of basic substances on oyster diastase.

Nature of basic substance	Sugar expressed as glucose found after 4 hours' incubation at 40° of oyster extract and 0·250 grm. of soluble starch	
	With water, mgs.	With basic substance, mgs.
Sodium carbonate 10 c.c. $\frac{N}{10}$	67·3	23·9
,, ,, ,,	51·8	16·4
Ammonia 10 c.c. $\frac{N}{10}$	49·3	16·7
,, 5 c.c. $\frac{N}{1}$	49·3	9·6

In association with the last two observations, two corresponding portions were, after incubation for two hours with the ammonia, acidified with acetic acid and incubated again for four hours. The figures respectively obtained were 50·9 and 21·8 in place of 16·7 and 9·6, the figures previously given. Another portion of the same extract was incubated straight off for four hours with acetic acid, and the figures given were 165·1.

The above results show that, as with invertase, the effect of the presence of a basic substance is to diminish enzymic action. It is also, in proportion to the amount present, damaging to the enzyme, so that, as exemplified in the last paragraph, its power fails to be restored, on treatment with an acid, to the state previously existing.

From these experiments on the oyster, passage was made to the liver in the higher animals, where the experimentalist is placed in a more difficult position through the rapidity with which enzymic change occurs as a consequence of the higher temperature existing.

A considerable amount of experimental work has been conducted upon the liver of the rabbit, and of the cat, in its ordinary *post-mortem* state, and likewise similarly upon the liver of the pig, calf, and sheep. The deduction to be drawn from this work is that in the state named a diastatic enzyme is present in a replete or fully activated form. Under these circumstances, enzymic power is not increased by the addition of an acid—indeed, as is generally known, a small addition suffices to produce an opposite effect. As a matter of fact, there is an optimum quantity concerned in the question, and when this quantity is passed, an inhibiting effect is produced running in accord with the quantity present.

Whilst ordinarily, then, under the circumstances named, incubation in association with added acid is not attended with the effect that has been described in the case of the oyster, some instances have nevertheless been observed to occur in which a certain amount of increased enzymic action has been noticed as an accompaniment of the acid addition. Apparently in these cases it may be taken that full activation had not existed.

The step now taken was to try the effect of treatment with a basic substance, under the idea of taking away the activating acid, and then making an acid addition to restore activity.

Fresh pig's liver was ground up with sand and water, and a strained-through-linen extract obtained.

To 20 c.c. of this extract (equivalent to 5 grms. of liver) it was thought advisable to add 500 mgs. of soluble starch instead of depending

upon the variable presence of glycogen. The incubating volume was made to 40 c.c. and incubation carried on for four hours at a temperature of 40°. After allowing for the sugar present in the extract at starting, it was found that the sugar produced, expressed as glucose, amounted to 266·9 mgs.

To another 20 c.c. of the extract treated in exactly the same manner, an addition was made of 10 c.c. $\frac{N}{10}$ solution of sodic carbonate. At the end of the incubation period, the amount of sugar produced was found to be decreased to 95·4 mgs.

A third 20 c.c. portion was treated with the 10 c.c. sodic carbonate solution and kept in contact with it for half-an-hour at room temperature. It was then just acidified with acetic acid and, similarly to the others, submitted to incubation. The sugar produced amounted to 256·2 mgs., a quantity which stands in pretty close accord with that belonging to the first named result.

In another similarly conducted experiment with a different specimen of pig's liver and with the employment of ammonia instead of sodic carbonate, the results came out as follows:—

After simple incubation with water	203·3 mgs. of sugar.
After incubation in the presence of 10 c.c. $\frac{N}{10}$ ammonia ...	43·8 mgs. of sugar.
After incubation of the product which had been in contact with 10 c.c. $\frac{N}{10}$ ammonia for half-an-hour at room temperature and was then acidified	207·7 mgs. of sugar.

In the subjoined experiment a modified course was pursued. The liver taken consisted of a portion of a cat's liver which had been placed instantly after death in a freezing mixture of ice and salt with a view of checking *post-mortem* change. On being removed from the freezing mixture, it was cut into thin slices and immersed in alcohol from which it was afterwards taken, pounded in a mortar, washed with alcohol, dried spontaneously, reduced to a minutely divided state, and brought to uniformity by passage through a fine sieve. 1 grm. portions were incubated with 100 mgs. of soluble starch in a 20 c.c. volume for two hours at 40°.

Incubated with water, the sugar produced amounted to	52·6 mgs.
,, ,, + 2 c.c. $\frac{N}{10}$ ammonia, the sugar produced amounted to	24·6 ,,
,, ,, + 4 ,, ,, ,, ,, ,,	13·3 ,,

Corresponding portions to the two latter which, like these, had been incubating with the ammonia for two hours, were just acidified with acetic acid and incubated further for two hours. The figures for the sugar produced that were now obtained were respectively 60·7 and 58·9, showing that, in the presence of the acid, full enzymic activity was restored.

Introduction of sodic carbonate into the liver by injection into the mesenteric vein.

Experimentation was now carried to the introduction of sodic carbonate into the liver of the living animal, and afterwards observing the extent of enzymic action occurring under varied conditions.

Cats have constituted the animals experimented upon. In a fully anæsthetised state, a cannula has been ligatured into the mesenteric vein just below its junction with the veins that throw themselves into the portal trunk. The object in view was to quickly run the solution through the vessels of the liver for the tissue to become everywhere permeated with it. About 250 c.c. has been found to be a convenient quantity to employ, and this was put into a glass vessel provided with a couple of openings, one serving for the exit of the fluid and the other for connection with a rubber spray pump by which the requisite pressure for the quick penetration of the liver is satisfactorily supplied. It is to be understood that in all experiments where the sodic carbonate is spoken of reference is made to the anhydrous salt.

The effect of sodic carbonate on the liver is to counteract the transformation of glycogen into sugar. It thus, like the effect of freezing, prevents the *post-mortem* production of sugar, and enables it to be readily seen that, as regards the presence of sugar, the liver, at the moment of death, stands in the same position as the other organs and tissues of the body.

The following is a record of an experiment in which a 5 % solution of sodic carbonate was used. The quantity in this case injected into the mesenteric vein amounted only to between 50 and 60 c.c. The left lobe of the liver had previously been ligatured off from the rest to supply a representation of the liver not subjected to injection. In this portion there was nothing to interfere with the ordinary *post-mortem* production of sugar taking place. 10 grms. of it were, a short time after death, ground up with sand and water and afterwards boiled as the first step in the treatment for the extraction of sugar by successive washings

and squeezings. It was found to contain 202·7 mgs. of sugar, which gives a per mille proportion of 20·27. Another 10 grms. treated in the same manner and incubated for four hours at 40° contained 434·4 mgs. of sugar, representing 43·44 °/₀₀.

The effect of the injection is to render the liver pale from the removal of its blood. The liver is seen to become tensely distended, and it is kept in this state for a short while before cutting through its vessels for effecting its removal. It then shrinks from the escape of the injected fluid.

In the experiment 10 grms. were taken for the sugar determination and extracted with boiling water, the alkalinity, as in all like cases, being removed by the addition of acetic acid to prevent the destruction of sugar. The amount of sugar found to be present was 23·1 mgs., which gives a ratio of 2·3 °/₀₀.

Another 10 grms., after being ground up with sand and water, were incubated for four hours at 40°. The amount of sugar here yielded was 32·9 mgs., which stands equivalent to 3·3 °/₀₀. As a result of the presence of the sodic carbonate, it is seen that only an insignificant amount of sugar had become produced during the incubation.

A third 10 grms., after being ground with sand and water, were just rendered acid with 3 c.c. of 10 °/₀ acetic acid, and then similarly incubated. The amount of sugar now found to be present was 129·3 mgs. or 12·9 °/₀₀.

A 2 °/₀ strength of sodic carbonate is equally as efficacious as a 5 °/₀ strength in arresting the *post-mortem* enzymic production of sugar as the following three experiments show. In each case the activating effect of subsequent treatment with acid is displayed.

Exp. 1. A full quantity of a 2 °/₀ solution of sodic carbonate was injected quickly into the mesenteric vein, the left lobe of the liver having previously been ligatured. 10 grms. of this lobe treated shortly after death for the extraction of sugar gave 166·7 mgs. = 16·7 °/₀₀ as the quantity present.

10 grms. of the injected liver, ground with sand and water and taken as a control, were found to contain 14·2 mgs. of sugar = 1·4 °/₀₀.

Another 10 grms., after incubation for two hours at 40°, contained 20·9 mgs. of sugar = 2·1 °/₀₀, an insignificant extent of rise.

A third portion of 10 grms. that was just acidified with acetic acid and then incubated for two hours at 40° contained 127·1 mgs. = 12·7 °/₀₀ of sugar.

Exp. 2. A corresponding method of procedure was adopted to that in the last, except that incubation was carried on for four hours instead of two, and that 300 mgs. of soluble starch were added to the products subjected to incubation to meet the possibility of a low amount of glycogen in the liver.

The 10 grms. portion of the ligatured liver taken about two hours after death contained 22·48 °/₀₀ of sugar.

The control, or non-incubated, portion of the injected liver yielded 1·45 ‰ of sugar; the corresponding incubated portion 2·98 ‰; and the incubated portion that had been previously just acidified (2·3 c.c. of $\frac{N}{1}$ acetic acid were required for the purpose), 16·1 ‰.

Exp. 3. This was carried out in a corresponding manner to the last, but was amplified by incubating with increasing quantities of acid.

10 grms. of the ligatured portion were taken for extraction with water about two hours after death. The amount of sugar found was 19·6 ‰.

Another 10 grms. after incubation for four hours showed the presence of 42·5 ‰ of sugar.

Experimentation with the injected portion was commenced about half-an-hour after death. The course of procedure adopted was designed to ascertain the amount of sugar present in 10 grm. portions of the liver taken at starting, after incubation alone, and after incubation with rising amounts of acetic acid. At starting the 10 grms. of liver were only found to contain 0·99 ‰ of sugar. After incubation alone for four hours at 40°, these figures only became raised to 2·6 ‰. The effect of the acid is shown in the subjoined tabular representation:—

Liver after injection with 2 % sodic carbonate incubated with rising quantities of acetic acid.

	Sugar found in 10 grm. portions, mgs.
At starting	9·9
Incubated alone	26·2
„ with 0·55 c.c. $\frac{N}{1}$ acetic acid ...	74·6
„ „ 1·1 „ „ ...	138·9
„ „ 2·2 „ „ (just acid)	151·5
„ „ 2·4 „ „ ...	135·1
„ „ 3·3 „ „ ...	123·5
„ „ 5·5 „ „ ...	71·5

It is seen that the effect of permeation of the liver with a 2 % solution of sodic carbonate is almost completely to hold in check diastatic enzymic action, even under exposure for four hours to a temperature of 40°. It may be mentioned parenthetically that it has been further found that even a 1 % strength suffices to arrest the *post-mortem* production of sugar that takes place in the liver allowed to remain under ordinary circumstances. Passing to the effect of the acid, the figures in the table show that a rising enzymic activity attends the rising addition of acid up to a certain point when the effect of a further addition is to occasion a progressive decline. The optimum point in the experiment from which the table is drawn is seen to be when the product just gave signs of the existence of acidity. When this point was reached, the next step of increase of acid was designedly only slight, and a certain amount of diminished production of sugar is noticed.

In the preceding experiments the moist liver substance was dealt with. The point that is being illustrated can be equally well exemplified upon liver that has been subjected to the coagulating influence of alcohol, and of acetone. The liver was well pounded in a mortar with the agent employed, and afterwards collected on a linen filter, squeezed, and washed two or three times. The product was then spread out and dried in the air and subsequently powdered, the portion used being that which had been passed through a fine sieve (90 mesh). 1 grm. portions were taken for employment.

The liver operated upon was derived from a cat into the mesenteric vein of which a 1 % solution of sodic carbonate had been injected.

The 1 grm. of the dry product derived from treatment with alcohol contained 5·5 mgs. of sugar to start with. Incubation as before was carried on for four hours at 40° with 20 c.c. as the incubating volume and with 200 mgs. of soluble starch present. After incubation with water, 16·4 mgs. of sugar were found, giving 10·9 mgs. as the amount of sugar produced. After incubation with 2 c.c. of $\frac{N}{10}$ acetic acid, which just rendered the product acid, 79·3 mgs. of sugar were found, giving 73·8 mgs. as the amount of sugar produced.

The 1 grm. of dry product derived from treatment with acetone, which, according to the experimental results that have been obtained, exerts a somewhat less damaging influence on the enzymic factor than alcohol, yielded the subjoined representation of produced sugar after treatment with acetic acid and with sodic carbonate:—

	Produced sugar, mgs.
Incubation with water	15·3
„ „ 1·35 c.c. $\frac{N}{10}$ acetic acid, which failed to overcome existing alkalinity ...	53·8
„ „ 2·7 „ „ which just produced a show of acidity	89·0
„ „ 1·35 „ sodic carbonate, which perceptibly increased the existing alkalinity ...	7·7

In the next experiment the liver was subjected to the acetone treatment after being taken in an ordinary way from a cat that had just been anæsthetised and killed. The procedure adopted was similar to that which has been described above.

	Produced sugar, mgs.
Incubated with water	106·2
„ „ 1 c.c. $\frac{N}{10}$ sodic carbonate	105·1
„ „ 3 „ „ „	78·8
„ „ 5 „ „ „	51·9
„ „ 10 „ „ „	20·2
„ „ 0·5 „ acetic acid	120·8

The liver here was in the ordinary *post-mortem* state, and is seen on incubation with water to have possessed considerable enzymic capacity which it may be assumed was given to it by *post-mortem* change. The 1 c.c. of $\frac{N}{10}$ sodic carbonate solution failed to produce any material effect on the existing capacity, but with the other quantities the occurrence of a successive fall is noticeable. The influence of the acetic acid is markedly perceptible, notwithstanding the smallness of the quantity that was employed.

The conclusion to be drawn from what has preceded, alike for invertase and diastase, is that enzymic activity is regulated by the character of the environment. The inactive state may be transformed into the active, and *vice versa*, without destroying the fundamental body concerned in the matter. The facts which have been adduced show that acids give activity, whilst bases hold activity under restraint; and that a very little alteration on either side suffices to determine whether a state of activity or of inactivity shall prevail. If only a weak state of acidity or alkalinity is brought into play, the dynamic body (zymogen) concerned does not show signs of being materially damaged, whilst actual damage is sustained in proportion as the strength of either factor is increased. The effect of a basic substance in its reducing influence on enzymic activity, and its damaging influence on the dynamic body, is greater in the case of invertase than in that of diastase.

Post-mortem acid production.

The effect of acidity has been shown in giving enzymic power to the dynamic substratum of diastase, which, from the facts presenting themselves, may be concluded to be present in the various organs and tissues of the body. Acidity thus constitutes an important factor in relation to the occurrence of enzymic action, and its production within the system becomes a matter of weighty significance.

It is known that an active production of acidity sets in immediately after death. The fact has long been recognised from study approached in different directions.

Fletcher and Hopkins[1] have carefully investigated the subject in the case of muscle. Their results have been based upon the estimation of lactic acid, and they have specially shown that a rapid development of it takes place in the recently excised muscle subjected to mechanical

[1] This *Journal*, xxxv. p. 247.

injury and to immersion in alcohol. They say that the production is attendant upon the survival state, but the fact exists that it occurs as a sequence of somatic death, and in the occurrence of the phenomenon the capacity of production is made manifest.

Magnus-Levy[1] has given attention to the acid production occurring in the liver as a part of the phenomenon of autolysis. His experiments clearly demonstrate the fact of acid production taking place as a *post-mortem* event, but they are not of a nature to show the rate at which it occurs after the destruction of life. Experimental evidence upon this point will be now adduced.

In the experimental investigation that has been conducted, it is the total acidity from all sources, measured by a colour indicator, that has constituted the matter dealt with. As solvent, alcohol (96 %) has been found to give much higher results[2] than acetone, which has been tried, and boiling water. With acetone the yield has been only about half, and with boiling water it has been found as an issue of several experiments to vary from one-third to one-seventh. Simple mention of these facts is here made with the remark that in connection with what follows alcohol has constituted the solvent employed, and it need hardly be stated that the alcohol used was ascertained to be free from action on the colour test. Sand was made use of to facilitate extraction, and straining through linen and washing entered into the process.

Phenolphthalein has been found to constitute an advantageous indicator, being easy to work with and giving a sharp end-point. Litmus has been tried, but with red and blue to deal with, the transition point is somewhat difficult to read off. It is known that there is a want of uniformity in the response of different indicators to weak acids, and this is perspicuously shown in connection with the work that is under consideration. With a preparation, for instance, from a particular animal product, phenolphthalein may give an acid, whilst litmus may give an alkaline, reaction. It is conjectured that hydrogen and hydroxyl ions are concerned in the matter. The problem is an intricate one, but

[1] *Beitr. z. chem. Physiol. u. Path.* II. 261. 1902.

[2] Fletcher and Hopkins (*op. cit.*) comment on alcohol as an excitor of the production of acidity, and they say that the same excitation does not occur when the muscle is frozen and immersed in alcohol brought to a low temperature by a freezing mixture. This point has not been overlooked in relation to the high results obtained by extraction with alcohol, the question suggesting itself whether special excitation of production might play a part in connection with the matter. Put to the test of experiment, support was not given, by the evidence supplied, to the existence of grounds for such an idea being entertained.

there is no need here to enter into its consideration. The facts of the case are all that need be dealt with, and the state of matters may be exemplified by the following examples drawn from the collection that has been obtained.

Illustrative examples of the difference in the reaction given by phenolphthalein and litmus.

	Extent of acidity and alkalinity indicated by c.c. of decinormal acid or alkali present in 100 grms. of the product employed	
	Phenolphthalein	Litmus
Cat's blood	10·0 acidity	3·75 alkalinity
,, spleen	55·6 ,,	3·5 ,,
,, kidney	37·4 ,,	Neutral
,, liver	42·5 ,,	2·5 acidity
Rabbit's blood	13·3 ,,	3·3 alkalinity
,, liver	43·4 ,,	Neutral
,, muscle	51·0 ,,	9·0 acidity

The object of the work under consideration is to show the alteration that sets in with the passage from the *ante-mortem* to the *post-mortem* state. Such being the case, we need not concern ourselves about the results yielded by different indicators but may take those yielded by phenolphthalein which is found to be the most satisfactory for employment.

As the following table shows, evidence of acidity is presented in all the examinations that have been made. To varying extents acidity is noticeable in the products taken shortly after death, and in all cases, excepting the blood, the acidity is notably greater on the following day.

	Acidity expressed as c.c. of $\frac{N}{10}$ acid per 100 grms. of product							
	Blood		Liver	Muscle	Kidney	Spleen	Pancreas	
Cat:	(1)	(2)					(1)	(2)
At once	10·0	9·2	42·5	53·3	37·4	55·6	230·0	100·0
Following day	8·75	9·3	92·5	75·8	69·4	100·7	—	113·9
Rabbit:								
At once		13·3	43·4	51·0				
Following day		14·0	71·2	78·2				

A fluoride is known to be a strong inhibitor of enzymic action, and upon the possibility that it might inhibit the *post-mortem* production of acidity, the following experimental inquiry applied to the liver was undertaken. The procedure adopted was to inject the fluoride solution into the mesenteric vein of an anæsthetised cat, a lobe of the liver having been previously ligatured to supply a counterpart portion of liver

for comparison. Increasing strengths of solution were used, and as water only takes up 4 % of sodium fluoride, the potassium salt had to be brought into use, and was employed, in fact, in all the subjoined experiments with the one exception of that falling under the 4 % strength. The distension of the liver which takes place at the time of the injection quickly subsides when the vessels are cut through, and it may be considered that no vitiating influence upon this ground is produced on the result by the injection. Phenolphthalein is not affected by the fluoride.

Effect of injection of fluoride solution into liver in relation to the production of acidity.

Strength of fluoride solution, %	Acidity expressed in c.c. of $\frac{N}{10}$ acid per 100 grms. liver in	
	Injected liver	Non-injected liver
2	25·7	37·4
4	22·5	45·0
8	19·5	45·0
10	16·7	41·7
20	18·1	38·8

From this table it is to be inferred that the production of acidity sets in with activity in the liver immediately after death. Evidence is afforded that a restraining influence is exerted by the fluoride solution, much lower figures being given under its presence than where *post-mortem* action was allowed to come into unchecked play; and, with the single exceptional instance at the bottom of the list, a progressively decreasing effect is seen to have followed the increasing strength of the solution.

The same sort of occurrence takes place as with the *post-mortem* production of sugar, and, seeing the position in which acidity stands in relation to amylolytic enzymic activity, it may be that the development of acidity constitutes the precursor and source of the sudden sugar formation that is observed to occur. Whilst natural *ante-mortem* conditions are existing, the circumstances may be such as to lead to the zymogen present in the cells escaping activation and then suddenly becoming activated by the instrumentality of acid development. There is nothing to be wondered at in the occurrence of a sudden change of state in relation to acidity, and with the altered state thus occurring, there is equally nothing to be wondered at in the sudden arrival of the aptitude for the conversion of zymogen into enzyme; and, following upon this, the sudden arrival of the capacity for the transformation of

glycogen into sugar. As long as zymogen remains in the state of zymogen, no capacity exists for the production of sugar, but with altered circumstances in relation to acid production, a sudden arrival of the capacity may be brought about.

The same line of reasoning may be applied to the diastatic enzymic power which is found to exist in the various constituents of the body after death. Because after death this power should recognisably exist, it is not to be taken that such should be the case during life. Circumstances become altered by the development of acidity that takes place as a *post-mortem* event. Inferences drawn from *post-mortem* conditions are exposed to the liability of leading to error if made to apply to the phenomena of life.

Plunging liver substance into a freezing mixture immediately after the destruction of life supplies evidence running, as far as it goes, in the same direction as that which has been already adduced. In the case of a rabbit's liver dealt with in this way, the acidity, expressed in c.c. of decinormal acid per 100 grms. of liver, was found to stand at 32·5 for the frozen against 43·3 for the non-frozen portion. With a cat's liver similarly treated, the figures were respectively 39·9 and 45·4. With a cat's liver where the frozen portion was extracted with alcohol specially circumstanced for being kept at a low temperature, the figures were 32·0 and 45·3. With a cat's liver plunged into boiling water instead of a freezing mixture, the figures given were 39·0 against 57·0 for a portion of the liver dealt with in the ordinary way.

As shown above, activity is determined by the state of the environment. With the metabolic changes into which enzymic action enters sequences occur from the demand existing. Inactivity passes into activity as the demand arises for the change, and circumstances reasonably point to the environment constituting the medium through the instrumentality of which the issue is evoked. For instance, in the growing seed, immediately circumstances lead to a demand for nutritive material by the embryo, the store of starch, by enzymic activity, becomes placed in a position to yield what is wanted. Whilst the zymogen, existing in connection with the embryo, remains in the state of zymogen, there is nothing to evoke enzymic activity. What is needed is the production of an activating agent, and this, it is to be considered, occurs with the first changes that take place under the influence of exposure to the physical conditions that start germination. The experiments of Brown and Morris[1] on barley grains are of deep interest as exponents

[1] *Journ. Chem. Soc.* LVII. 458. 1890.

of this matter. These authors noticed that the detached embryo, placed upon an admixture of starch and gelatine, appropriated the starch to its growth just as occurs within the grain. To do this, the precursor of the enzyme, which they found located in a layer of subtegumentary cells, must necessarily become activated, and, in view of what has preceded, the simple formation of acid may be taken as sufficient for the purpose. Obviously it must be from the embryo that the operative agency is derived.

The influence of demand in calling forth the operative procedure is strikingly exemplified in further experiments of Brown and Morris in which they placed the barley embryos in contact with cane-sugar and starch. They found that the cane-sugar protected the starch from enzymic attack. As long as cane-sugar was present, it was used up in preference to the starch. Assumedly the diastatic factor was, in the meantime, resting in an inactivated state, and only became activated when the want of starch set in. Analogous illustrations can be drawn from the animal system and notably from the liver in relation to glycogen. Evidence shows that under stress of requirement, glycogen is drawn upon and removed in a manner that does not under other circumstances occur, and the facts to be dealt with reasonably permit of the assumption that the demand evokes an alteration which leads to an activation of the diastatic zymogen present in the hepatic cells.

Summary.

The inverting power of an aqueous extract of yeast is increased ten to fifteenfold by the addition of a boiled extract of yeast. This activating effect is shown to be attributable to the acidity of the boiled extract, equivalent results being obtainable by the substitution of acetic acid for the boiled extract.

Yeast cells may be used in the place of the aqueous extract, and with increasing additions of acetic acid, an increasing activating effect is produced until an optimum point is reached after which the further addition of acid leads to a diminution of inverting power.

After desiccation, treatment with alcohol, or grinding up with kieselguhr, yeast yields an aqueous extract which possesses greatly enhanced enzymic power, and which, unlike the ordinary extract, fails to show more than a comparatively small increase of power on acid addition.

The activity given by acid addition is susceptible of removal by a basic substance. Ammonia, sodic carbonate, etc., and even the basic

material of tap-water, exert an inhibiting influence which the subsequent addition of an acid is capable of counteracting. Under one form of environment the power is lost, and when changed to another, the power is restored.

The diastatic enzymic agent in the oyster is influenced by boiled oyster extract and by acetic acid after the same manner as occurs with invertase, but not to so great an extent. The effect of acid addition is not only to increase the amount of cupric oxide reducing product but to carry it into a higher cupric oxide reducing state.

In the case of the higher animals, the liver when taken in the ordinary *post-mortem* state, in which a fully-activated enzyme exists, has been usually found not to be affected like the liver of the oyster by acid addition, but as a rule to have its enzymic power diminished.

As with invertase, basic substances exert an inhibitory influence over diastase, and the inhibitory effect can be removed by acid addition, thereby leading to the restoration of active power.

The *post-mortem* production of sugar in the liver may be virtually checked by the influence of sodic carbonate injected in a 2 %, or even less, strength of solution into the portal system of the living animal. The addition of rising quantities of acetic acid to the liver thus treated brings about a rising possession of enzymic power till an optimum is reached, after which the further addition leads to a decline of power.

The liver after coagulation with alcohol or acetone and subsequent desiccation gives the same response, in relation to enzymic action, to the influence of acid and alkali as the fresh organ.

Under the head of *post-mortem* acid production evidence is adduced showing the rapidity and magnitude of its occurrence in the liver and other organs of the body, and that in the liver it presents a similitude to the *post-mortem* production of sugar, of which it may possibly be the precursor.

The mode of quantitatively estimating the acidity is entered into, and the different positions held by phenolphthalein and litmus as indicators shown.

A restraining influence is exerted by a fluoride on *post-mortem* acid production in the liver in like manner as occurs with *post-mortem* sugar production.

Consideration is finally given to the bearings of acid production on enzymic action and metabolism.

THE MODE OF UNION OF CERTAIN POISONS WITH CARDIAC MUSCLE.

By H. M. VERNON.

(*From the Physiological Laboratory, Oxford.*)

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In previous papers[1] I described the action of numerous poisons upon the gaseous metabolism of the mammalian kidney and the tortoise heart, the object of the experiments being to obtain some information as to the probable nature of the loose combinations temporarily formed by the poisons with the living tissues. I have now endeavoured to attack the same problem, but in a different manner, by systematic investigation of the action of graduated concentrations of certain poisons upon the beating tortoise heart. The heart of *Emys europœa* was used in all but a few control experiments, which were made with the heart of *Testudo græca*. By means of a cannula tied in the aorta, the heart was perfused with oxygenated Ringer's solution containing ·75 % NaCl, ·024 % $CaCl_2$, ·021 % KCl and ·01 % $NaHCO_3$, generally at a pressure of 40 to 70 cm. of water. The heart hung in a beaker of saline, and this beaker was supported in a water bath, the temperature of which was kept approximately constant at 18° C. throughout the experiment by the

[1] Vernon. This *Journal*, xxxix. p. 149. 1909; xl. p. 295. 1910.

radiation from a glow lamp. Immediately previous to perfusion of the heart with the poison-containing Ringer's solution, the saline in the bath was syphoned off and replaced by some of the same poison solution, so that the cardiac muscle was subjected to its action from every side at the same time. This precaution is necessary, for diffusion from the surrounding saline into the cardiac tissue is very rapid, so much so that by employing about four times the concentration of poison one can get almost the same effects by diffusion from the outside as by perfusion through the coronary vessels. Also a heart will beat for many hours if kept in oxygenated saline, and not perfused at all, whilst on substitution of oxygen-free saline its beats sink to zero in about an hour.

The number of poisons investigated was six, three of which, viz. alcohol, ether and chloroform, are supposed by Meyer and Overton to act by first dissolving in the lipoids of the tissues, whilst the other three, viz. hydrocyanic acid, sodium fluoride and formaldehyde, probably enter into direct and definite chemical combination with certain of the tissue constituents. Hence one might expect that the course of action of these two groups of poisons would be different, and such proved to be the case.

The Action of Alcohol.

In the experiments with ethyl alcohol, pure absolute alcohol was added to oxygenated Ringer's saline so as to make up solutions containing from ·5 up to 8 parts by weight per 100. Poisoning was continued for 20 to 30 minutes as a rule, the alcohol saline being perfused at the rate of about 1 c.c. per min. for each gram of heart substance. Fresh oxygenated Ringer's solution was then substituted, and the alcohol saline was syphoned off from the heart beaker, replaced by Ringer's solution, and this was in turn replaced by more Ringer's solution. The heart beats, magnified 14 times, were recorded on a smoked drum, and were subsequently measured. A typical result is shown in Fig. 1. Ordinates represent the contraction height in millimetres, and abscissæ the time in minutes. It will be seen that ·5 % alcohol reduced the beats to a constant level in about 14 mins. and for the remaining 16 mins. of the poisoning exerted no further effect. At the point marked *R*, the alcohol was replaced by Ringer's solution, and about a minute later the heart began to recover, and 10 mins. later had practically regained its initial contraction height.

Most of the apparent latent period between substitution of Ringer's solution and beginning of recovery is of instrumental origin. The cannula and junction T-piece had an internal volume of ·5 c.c., and so there was about ·5 to 1 min. delay between the substitution of the fresh saline, and its arrival at the heart.

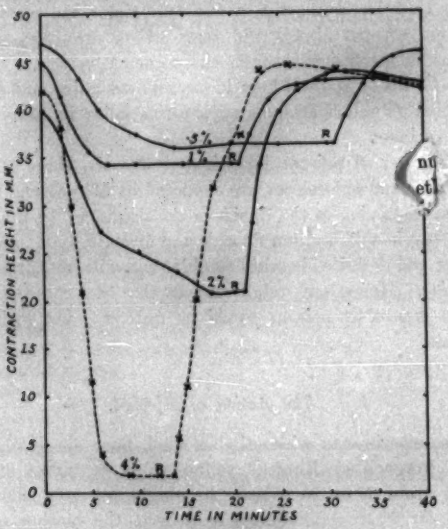

Fig. 1. Alcohol.

An hour and a half after the first poisoning, the heart was poisoned with 1 % alcohol, and with this concentration the beats were reduced to a constant height in about 7 mins. With 2 % alcohol constancy of contraction height was reached only after 18 mins., but with 4 % alcohol, in 9 mins. In every experiment, therefore, the alcohol depressed the cardiac contractions to a constant level, and then ceased to exert any further depressant influence. Presumably a definite amount of the poison, more or less proportional to its concentration, had entered into some sort of combination with the contractile substance of the cardiac tissue, and had thereby depressed its contractile power, or weighed it down. Once equilibrium was established, the loaded heart went on

beating at its lowered level of activity without further reaction to the alcohol. From the slope of the curves in the figure it will be seen that the rate of recovery of the heart was roughly the same in all four experiments, or presumably the alcohol broke away from the cardiac substance at the same rate.

As will be pointed out in due course, this action of alcohol in reducing the cardiac contractions to a constant equilibrium level is common to all the six poisons investigated, and doubtless it is true of many other poisons, though not of cumulative poisons such as acids and alkalis, and salts of the heavy metals. Some of the published records of the action of poisons on cardiac contraction show clear evidence of it[1], but as far as I am aware it has been investigated in detail, and its significance remarked on, only by Prof. Sherrington and Miss Sowton[2]. These investigators perfused cats' hearts with warm oxygenated Ringer's solution containing known amounts of chloroform, and they found that within a minute or two the contraction height was reduced to an equilibrium level, and would continue unchanged for as long as 20 mins. Full recovery occurred about 2 mins. after substitution of fresh Ringer's solution, and Prof. Sherrington and Miss Sowton believe this to show that "the chloroform behaves as though it were in solution in the muscle," and they say, "Can this drug poison the muscle without entering into chemical combination with its substance?"

Similar experiments to those above described were made at different times of the year, and the data given in the accompanying table indicate

Concentration of alcohol	April 3rd	April 8th	June 22nd	June 22nd (repeated)
·5 %	—	77 % (14 m.)	—	—
1	—	76 % (7 m.)	89 % (10 m.)	95 % (11 m.)
2	27 % (20 m.)	52 % (18 m.)	59 % (19 m.)	86 % (9 m.)
4	13 % (9 m.)	4 % (9 m.)	13 % (11 m.)	11 % (12 m.)
8	—	—	—	—

Concentration of alcohol	July 27th	July 27th (repeated)	Aug. 6th	Dec. 20th
·5 %	—	—	—	—
1	90 % (10 m.)	86 % (10 m.)	—	—
2	(52 % in 30 m.)	47 % (13 m.)	90 % (13 m.)	80 % (10 m.)
4	24 % (12 m.)	9 % (11 m.)	46 % (11 m.)	(48 % in 30 m.)
8	—	—	—	1·3 % (15 m.)

[1] Cf. G. R. Mines. This *Journal*, XL. p. 327. 1910.

[2] Sherrington and Sowton. *Thompson Yates and Johnston Laboratories Reports*, V. Pt. 2. 1903.

that the action of alcohol on the tortoise heart is not influenced by season. The figures in the table show the percentage height of the heart contraction on the initial height when equilibrium was attained, and—in brackets—the time in minutes required to reach equilibrium. In every experiment but two equilibrium was reached, generally in 9 to 13 mins. In the two in which the contraction height continued to fall throughout the 30 mins. poisoning, the percentage height at the end of the perfusion period is given. The susceptibility of different hearts to alcohol varies greatly, as 2 % alcohol reduced the contraction height by amounts varying from 90 % to 27 %, and 4 % alcohol by from 48 % to 4 %. In a single experiment on the heart of *Testudo*, 1 % alcohol reduced the beats to a constancy level of 47 % in 12 mins.: 2 % alcohol to one of 19 % in 20 mins., whilst 4 % alcohol reduced the beats to 3 % their initial height in 10 mins., but not to constant level. Only one of the several *Emys* hearts tested would beat when perfused with 8 % alcohol, and this heart was perfused five times with this concentrated poison for intervals of 5 to 20 mins., and only on the last occasion, after the heart had been perfused and experimented with for 46 hours, did the alcohol temporarily stop its beats. The effect of various poisons on the rate of heart beat will be discussed subsequently, but it may be stated here that alcohol is peculiarly liable to bring the heart to a sudden stop. It stopped it in 1 of the 9 experiments made with 1 % alcohol: in 2 out of the 12 with 2 % alcohol, and in 9 out of the 11 made with 4 % alcohol. The heart would always give a single beat on mechanical excitation such as a slight pinch of the forceps, and such excitation was always made in the experiments recorded in the above table. An actual record, obtained on a slow drum, is shown in Fig. 2. In this experiment, the heart ceased spontaneous beats after 5 mins. poisoning with 4 % alcohol, and except for two short intervals did not begin them again till 5 mins. after the alcohol saline was replaced with fresh Ringer's solution. All the contractions over which a dot has been made are the result of mechanical excitation. This record also illustrates the excitatory effect of alcohol, for on recovery the contraction height was 8 % greater than the initial height, and it was some minutes before the heart beats sank to their original level. In 38 out of the 46 records obtained a similar excitation was observed. After poisoning with 8 % alcohol, the contraction height on recovery was on an average 17 % greater than the initial height. After poisoning with 4 % alcohol it was 8 % greater: after 2 % alcohol, 8 % greater, and after 1 % alcohol, 5 % greater. Hence the excitation was the more marked, the more

concentrated the alcohol used for poisoning. This is an unexpected result, as one would have thought that 8 % alcohol would permanently injure the cardiac tissue. However, there was no clear evidence of injurious action in any of the experiments with alcohol. The small deterioration of contraction height observed during the course of the day's experiments (on an average about 20 % in 9 hrs.) was very little more than that shown by unpoisoned hearts. In the above table it will be seen that on two occasions the heart was poisoned a second time with each concentration of alcohol, the second series of observations being made on an average seven hours after the first series: but only in one instance was the heart distinctly more affected by the second poisoning than by the first.

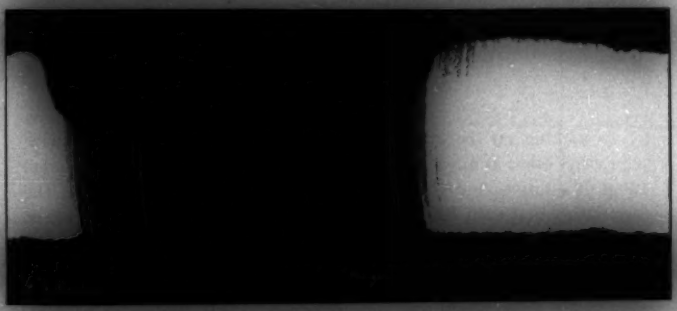

Fig. 2. × ½. Alcohol. Time in minutes.

Attention has already been drawn to the fact that the slope of the curves in Fig. 1 shows the rate of recovery of the heart to be about the same whatever the concentration of alcohol used; but in order to be able to compare the rate of recovery of different hearts, and of hearts subjected to different poisons, it is necessary to reduce it to numerical terms. The slope of the curves at the beginning of recovery is not very constant in form, nor is that of the final portion of the curves when recovery is almost complete; but the middle portion represents the rate of recovery with some accuracy, and so I calculated the time required by the hearts to raise their contraction height from a lower limit of 20 % of the height finally attained by them to an upper one of 80 % of this height. Of course this method of measurement excludes all experiments in which the poison was not strong enough to depress the

contraction height to less than 20 % of its value. Also experiments on hearts which had been perfused for more than 12 hours are rejected, for a reason to be explained further on. The recovery times of the remaining experiments are given in the table, and from them it will

Concentration of alcohol	Time of recovery of 20 to 80 % of contraction height	Mean
1 %	6, 6	6·0 mins.
2	9, 5, 6	6·7
4	4, 6, 6, 5, 5, 3, 5, 7	5·1
8	4, 4, 7, 4	4·8

be seen that in the great majority of experiments the rate of recovery was nearly constant. The mean values indicate that it was rather less in hearts poisoned with 4 and 8 % alcohol than in those poisoned with 1 and 2 %, but probably this result is due to insufficiency of data.

The Action of Ether.

In making up the ether solutions, Ringer's solution saturated with pure ethyl ether at about 18° was added in known amount to oxygenated Ringer's solution, and the concentrations given are calculated on the assumption that each 100 c.c. of ether saline contained 8 grams of ether. The action of ether is very similar to that of alcohol. Fig. 3 shows the effect of perfusion with saline containing from 1 part of ether in 480 to 1 part in 60. The weakest ether saline reduced the heart beats to a constant level in 20 mins., but on substitution of Ringer's solution the recovery was imperfect. The 1 in 240 ether reduced the contraction height to its minimum level in 4 mins., and for the remainder of the poisoning the beats increased somewhat in magnitude. Still greater was the increase observed in the next experiment, when 1 in 120 ether was used, for after 6 mins. poisoning the contraction height was reduced to 17 % of its initial value, whilst after a further 14 mins. of etherisation it had risen to 30 % of its value. Such increase of contraction height during the poisoning was observed with one of the four other hearts experimented with, the remaining three showing a constant level throughout as in the alcohol experiments. A repetition of the poisoning with 1 in 240 ether four hours later gave the usual result of constant level during the poisoning, so perhaps the increase observed on the first occasion was produced indirectly by excitation of the augmentor nerves of the heart.

The fourth curve in Fig. 3 shows the effect of 1 in 60 ether. By this concentration the heart was stopped after a minute, but it still beat on mechanical excitation for the next five minutes, and then it failed to beat at all. However, the concentrated ether did no permanent injury to the heart, as the rate of recovery, on substitution of Ringer's solution, was the same as in the other experiments, and the final contraction

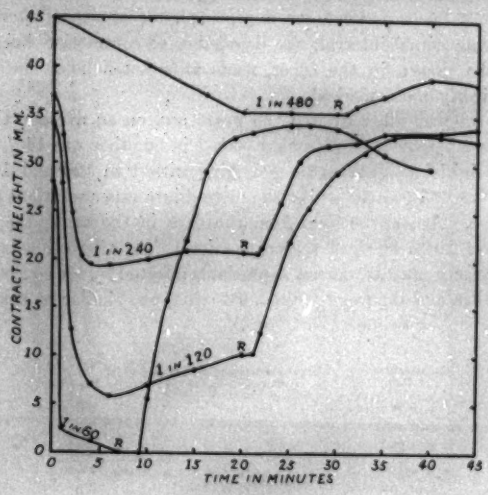

Fig. 3. Ether.

height of the heart was 21 % greater than the initial height. Other hearts proved less sensitive to the action of ether, for it will be seen from the table that in two of the other experiments the concentrated ether saline reduced the contraction height only to 33 and 37 % respectively. The other data in the table show the similarity of ether to alcohol. Constancy level was attained in 10 mins. on an average, as

Concentration of ether	April 4th	April 20th	April 20th (repeated)	June 24th	July 29th
1 in 960	80 % (10 m.)	90 % (10 m.)	—	—	—
1 in 480	56 % (9 m.)	83 % (4 m.)	—	(83 % in 30 m.)	77 % (20 m.)
1 in 240	—	73 % (5 m.)	52 % (12 m.)	76 % (10 m.)	53 % (4 m.)
1 in 120	28 % (16 m.)	44 % (9 m.)	42 % (13 m.)	48 % (8 m.)	17 % (6 m.)
1 in 60	5 % (10 m.)	37 % (10 m.)	—	33 % (8 m.)	(0 % in 7 m.)

compared with 12 mins. in the alcohol experiments, and the depression of contraction height was roughly proportional to the concentration of the ether. In those few experiments in which the contraction height rose gradually during the poisoning, after the initial depression, the constancy height given in the table is the lowest value attained during the poisoning. Only in two of the 19 experiments made did the contraction height gradually dwindle throughout the poisoning. On April 20th two of the experiments were repeated, and in one of them, made after a four hours' interval, the depression of contraction height was almost the same; in the other, made after a ten hours' interval, it was distinctly more marked.

As a rule the ether excited the heart, and on an average the final contraction height after poisoning with 1 in 60 ether was 14 % greater than the initial height. After poisoning with 1 in 120 and 1 in 240 ether it was 6 % greater, whilst the more dilute ether solutions scarcely affected it. As can be seen from the data in the table, the rate of recovery of 20 to 80 % of the contraction height after ether poisoning was fairly constant. It was not appreciably affected by the concentration of the ether, and the average time, 6·3 mins., was similar to that found in the alcohol experiments (5·4 mins.).

Concentration of ether	Time of recovery of 20 to 80 % contraction height	Mean time
1 in 960	6	6·0 mins.
1 in 480	7	7·0
1 in 240	7, 6, 8	7·0
1 in 120	7, 5, 8, 7	5·5
1 in 60	8, 7, 6, 5	6·5

The Action of Chloroform.

The action of chloroform is similar to that of alcohol and ether, in that it depresses the contraction height of the heart to a constancy level more or less proportional to the concentration of the poison, but it has in addition another property, which renders the experimental results unreliable. This is its well-known depressant action on cardiac muscle, which in the case of the tortoise heart is specially marked, and is to some extent permanent. A heart can be poisoned over and over again with alcohol, and give practically the same result each time, but a heart once poisoned with chloroform, though it may recover its initial contraction height when the poison is washed out with Ringer's solution, is

found to be depressed much more profoundly when poisoned a second or third time with the same concentration of the poison. Data in proof of this statement will be given in a subsequent section of this paper, but Fig. 4 gives an instance of the kind of result one obtains. In this experiment the fresh heart was first poisoned for 30 mins. with 1 in 16,000 chloroform saline (it being assumed that saline saturated with chloroform at 18° contains 1 part in 200). Constancy was attained in 13 mins., and the heart beat steadily for the remaining 17 mins. The

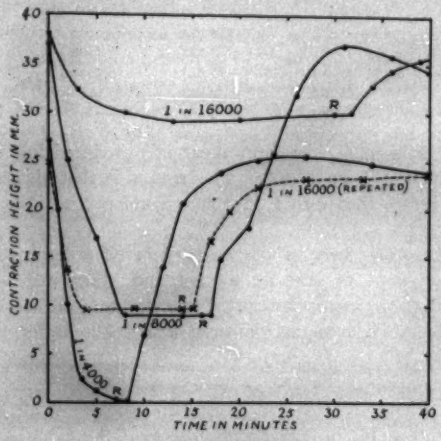

Fig. 4. Chloroform.

heart was then poisoned with 1 in 8000 chloroform. This produced a much more marked depression, and constancy was attained in 8 mins. The 1 in 4000 chloroform used next stopped the spontaneous beats of the heart in 2½ mins., and reduced the response to mechanical stimuli almost to zero in 7 mins. Half an hour later the heart was again subjected to 1 in 16,000 chloroform, and the dotted line curve given in the figure shows the result obtained; *i.e.* the chloroform depressed the contraction height of the heart to a constancy level of 38 % the initial height, instead of to the previous level of 79 %. A third poisoning of the heart with 1 in 16,000 chloroform 2¾ hours later depressed the contraction height to 22 % the initial height.

From the slope of the curves it will be seen that the rate of recovery was about the same in all the experiments. Also the heart always regained very nearly its initial contraction height.

From the data given in the table, it will be seen that constancy of contraction height was attained in almost all the experiments, and the time of attainment—averaging 12 mins.—was about the same as that found for alcohol and ether. Each result marked with an * is the first of the series, made upon a fresh heart. In the experiments made on

Concentration of chloroform	April 2nd	April 22nd	June 25th	Aug. 4th
1 in 32000	—	66 % (12 m.)	—	*81 % (8 m.)
1 in 16000	—	53 % (13 m.)	*79 % (8 m.)	68 % (14 m.)
1 in 8000	14 % (2 m.)	*67 % (11 m.)	24 % (8 m.)	16 % (30 m.)
1 in 4000	*5 % (23 m.)	2 % (9 m.)	(1 % in 7 m.)	(0 % in 8 m.)
1 in 2000	(·7 % in 5 m.)	—	—	—

April 22nd, 1 in 8000 chloroform apparently produced less effect than 1 in 16,000 and 1 in 32,000. The reason is that this experiment was made before that with 1 in 4000 chloroform, and the other two after it.

The rate of recovery of the heart from chloroform poisoning is probably the same as that for alcohol and ether, and is similarly independent of the concentration. The average of all the data given in the table is 6·6 mins., as compared with 5·4 mins. for alcohol, and 6·3 mins. for ether.

Concentration of chloroform	Time of recovery of 20 to 80 % contraction height	Mean
1 in 16000	6	6·0 mins.
1 in 8000	10, 8, 5, 5, 9	7·4
1 in 4000	6, 7, 5, 5, 6, 4, 11	6·3
1 in 2000	6, 6, 6	6·0

The final contraction height of the heart was on an average 2 % greater than the initial height after poisoning with 1 in 4000 chloroform: 7 % greater after 1 in 8000: 3 % greater after 1 in 16,000, and 4 % greater after 1 in 32,000. Hence the depressant effect did not appear immediately, but the heart, after showing a perfect recovery, gradually deteriorated in contraction height at a more rapid rate than that experienced when alcohol, NaF and HCN were used, though, unexpectedly, not so rapidly as after ether.

It is of interest to compare the concentrations of alcohol, ether and chloroform which produce similar effects on the tortoise heart, though the chloroform data are not very satisfactory. Taking only the chloroform

experiments made with fresh hearts, and taking means of the constancy levels given in previous tables for alcohol and ether, we get the following data:

Concentration of chloroform	Constancy level	Concentration of ether	Constancy level	Concentration of alcohol	Constancy level
1 in 32000	81 %	1 in 960	85 %	1 %	87 %
1 in 16000	79	1 in 480	75	2	60
1 in 8000	67	1 in 240	63	4	17
1 in 4000	5	1 in 120	33		
		1 in 60	16		

The depression of contraction height to 67 % produced by 1 in 8000 chloroform would be effected by about 1 in 320 ether, and by 1·75 % alcohol, or 1 part of chloroform would be equivalent to 25 of ether, and to 140 of alcohol. Otherwise expressed, chloroform is 140 times more active than alcohol, and ether 5·6 times more active. Dieballa[1], who perfused frogs' hearts with Albanese's sodium chloride and gum solution, found that the relative amounts of the poisons required to bring about arrest of the heart were 1 of chloroform, 23 of ether, and 75 of alcohol. Waller[2], working with the sartorius muscle, found that the contraction height was depressed to the same extent by 1 of chloroform, 8 of ether, and 40 of alcohol, whilst Waller and Symes[3] observed that the blood-pressure of the cat was lowered by about the same amount on intravenous injection of the poisons in the proportions mentioned. Evidently, therefore, the heart muscle both of the frog and the tortoise is relatively more sensitive to chloroform than is the sartorius muscle. Still, no accurate comparison of the poisons is possible, as the ratio of their activity varies greatly at different concentrations. For instance, the above data indicate that to reduce the contraction height of the heart to 16—17 % the initial height, requires relatively 2·4 times more alcohol than ether, but to reduce it to 85—87 % requires relatively 9·6 times more. An intermediate reduction to 60—63 % required 4·8 times more.

The Action of Hydrocyanic Acid.

The characteristic action of hydrocyanic acid on the tortoise heart can only be demonstrated by observing the effects of a wide range of

[1] Dieballa. *Arch. f. exp. Path. u. Pharm.* XXXIV. p. 137. 1894.

[2] Waller. This *Journal*, XXXVII.; *Proc.* p. LXXI. 1908, and *Proc. Roy. Soc.* LXXXI. B, p. 545. 1909.

[3] Waller and Symes. *Q. J. Exp. Physiol.* III. p. 115. 1910.

concentrations of the poison. As can be seen from the curves in Fig. 5, perfusion with ·0001 % HCN reduced the heart beats slightly during the first 20 mins., and then constancy was maintained for the remaining 20 mins. of perfusion. Experiments on other hearts with ·0003125 and with ·000625 % HCN were found to bring about more or less proportionate depressions of contraction height to a constancy level, but they were not

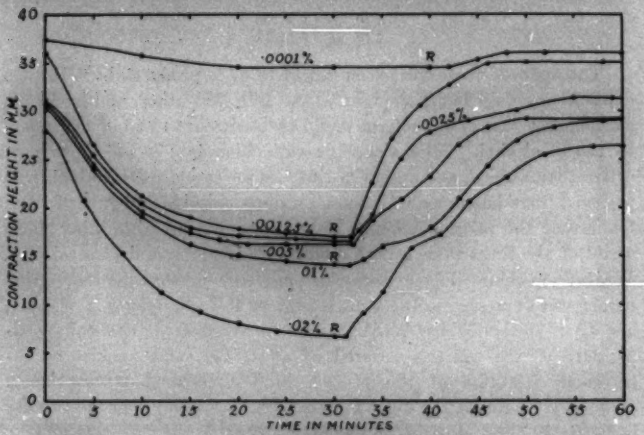

Fig. 5. Hydrocyanic acid.

made on the heart under discussion. On perfusing it with ·00125 % HCN, however, it will be seen that the contraction height was gradually depressed throughout the 30 mins. poisoning, without equilibrium being attained. Subsequent perfusions with ·0025 % HCN and with ·005 % HCN gave very similar curves, except that with the latter concentration constancy of contraction height was attained after 21 mins. Even ·01 % HCN depressed the cardiac contractions very little more than ·00125 % HCN, and so it follows that over a certain range of concentration the effect produced is *not proportional to the concentration*. The curves suggest that a definite amount of HCN tends to become bound up in the tissues, whether its concentration in the perfusion saline be ·000125 % or ·01 %, the only difference being that it takes rather longer for the heart to attain a constant contraction height with the weaker solutions than with the stronger. With still greater concentrations, the heart

beats are depressed further, more or less in proportion to the concentration. This fact is well shown by the curves in Fig. 6, obtained with another heart. They show that ·0025 % and ·005 % HCN reduced the beats practically to the same level, whilst the greater concentrations produced proportionately greater depressions, just as do alcohol, ether and chloroform. However, there is one important difference in the effects produced, for it will be noticed that there was no tendency for the heart to reach constancy of contraction height during the poisoning, and on washing out the poison with fresh Ringer's solution, the heart recovered at a slower and slower rate the greater the concentration, and moreover it never regained its initial contraction height. That is to say, concentrations of HCN above ·005 or ·01 % seem to injure the heart permanently, so that the increasing depression of contraction height produced by the greater concentrations is at least in part an injury effect.

The table shows the results of the experiments made upon the heart of *Emys*.

Concentration of HCN	March 29th	April 1st	April 2nd
·0025 %	—	—	—
·005	15 % (6 m.)	—	27 % (15 m.)
·01	—	14 % (14 m.)	—
·02	(7 % in 20 m.)	—	(1 % in 20 m.)
·04	(0 % in 6 m.)	(3 % in 10 m.)	—

Concentration of HCN	April 7th	July 18th	July 18th (repeated)
·0025 %	(50 % in 30 m.)	26 % (26 m.)	34 % (15 m.)
·005	57 % (5 m.)	33 % (19 m.)	35 % (15 m.)
·01	(39 % in 20 m.)	(29 % in 30 m.)	(30 % in 30 m.)
·02	(29 % in 15 m.)	(11 % in 30 m.)	—
·04	(8 % in 10 m.)	(4 % in 15 m.)	—

Concentration of HCN	Aug. 8th	Aug. 22nd	Dec. 16th
·0001 %	92 % (20 m.)	—	—
·0003125	—	78 % (23 m.)	—
·000625	—	59 % (20 m.)	—
·00125	(45 % in 30 m.)	(52 % in 30 m.)	—
·0025	(53 % in 30 m.)	(48 % in 30 m.)	—
·005	53 % (20 m.)	43 % (25 m.)	—
·01	(46 % in 30 m.)	26 % (24 m.)	—
·02	(24 % in 30 m.)	—	14 % (23 m.)
·04	—	—	(1 % in 20 m.)

As in previous tables, the results of experiments in which constancy was not attained are enclosed in brackets. All of the poisonings

with ·005 % HCN reduced the heart to constancy level, but as already explained, greater concentrations than this generally failed to do so as they injured the heart, whilst smaller concentrations were often not strong enough to do so in the 30 mins. over which the poisoning extended. The average constancy time in the experiments with ·005 % HCN was 15 mins., and in those with ·0025 % or less, 21 mins.

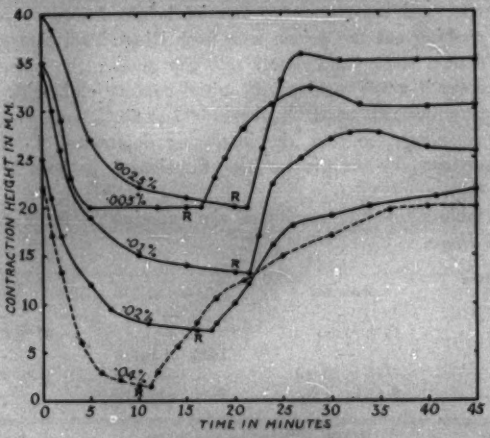

Fig. 6. Hydrocyanic acid.

The non-proportionality of the effects produced is shown in five of the sets of data given, the others not being complete enough for the purpose. In the experiments of April 7th ·0025 and ·005 % HCN had about the same effect on the heart. In those of July 18th ·0025, ·005 and ·01 % had the same effect, whilst ·02 and ·04 % had a much more marked one. Nevertheless on repetition of the poisonings with ·0025 to ·01 % HCN some 9½ hours after the first set of experiments, the heart beat was again reduced practically to the same relative level as before. It was not the same absolute level, as the previous poisoning with ·02 and ·04 % HCN had depressed the contraction height of the heart to 68 % of its original height. In the experiments of Aug. 5th (reproduced in Fig. 5), ·00125 to ·01 % HCN had practically the same effect, whilst in those of Aug. 22nd ·00125 and ·0025 % had nearly the same effect, and ·005 % a slightly greater one. In every case, therefore,

similarity of effect was produced by ·0025 and ·005 % HCN, and also by ·00125 % HCN when this concentration was investigated, whilst ·01 % HCN caused the same effect in three out of the five sets of experiments. In three out of the five sets also the heart beats were depressed by these concentrations to about 50 % of their initial height, but in the remaining two sets, to 30 % of their height.

Unfortunately I was unable to repeat these experiments on the heart of *Emys* as I could not obtain more material, but two experiments made on the heart of *Testudo* indicated a similar want of proportionality between concentration of poison and effect produced. The concentration of poison required to reduce the contraction height to constancy was almost twice as great as with the heart of *Emys*, for in the first experiment ·01 and ·02 % HCN lowered it to about 69 % the initial height,

Concentration of HCN	Heart of *Testudo*	
·0003125 %	—	95 % (11 m.)
·000625	91 % (12 m.)	84 % (13 m.)
·00125	90 % (12 m.)	80 % (9 m.)
·0025	(81 % in 30 m.)	72 % (20 m.)
·005	(69 % in 30 m.)	64 % (8 m.)
·01	67 % (22 m.)	64 % (8 m.)
·02	70 % (15 m.)	51 % (12 m.)
·04	—	(2 % in 12 m.)

and ·005 %, reduced it to the same level, though the 30 minutes' poisoning was not long enough for the attainment of constancy. In the second experiment ·005 and ·01 % HCN produced the same effect, but otherwise the depression of contraction height was more or less proportional to the concentration. This experiment was remarkable in that the heart beats were reduced to constancy level by seven out of the eight strengths of poison investigated.

The heart of *Testudo* is probably not so suitable as that of *Emys* for investigating the action of poisons, as it more frequently ceases spontaneous beats. Thus in the first of the above experiments ·005 to ·02 % HCN stopped the heart for 6 to 27 mins., though it always readily responded to pinching, whilst in the second experiment ·01 % HCN stopped it for 16 mins., though ·02 and ·04 % HCN did not stop it at all.

It will be seen from the slope of the curves in Figs. 5 and 6 that the rate of recovery of the heart after HCN poisoning was slower and slower the greater the concentration of the poison, even when the weaker solutions were used, and much more so with the stronger solutions. Of

the other experiments, an *Emys* heart gave curves with the same slope after being poisoned with ·0003125 to ·00125 °/₀ HCN, whilst one *Testudo* heart gave similar curves after poisoning with ·000625 to ·005 °/₀, and the other one after ·000625 to ·025 °/₀ HCN: but presumably as a rule even the weaker HCN solutions slightly reduce the vitality of the heart. The more marked influence of the stronger solutions is well shown by the 20 to 80 °/₀ recovery times given in the table, for it will be seen that after poisoning with ·04 °/₀ HCN the rate of recovery was on an average three times longer than after ·0025 to ·01 °/₀ HCN.

Concentration of HCN	Time of recovery of 20 to 80 % contraction height	Mean time
·0025 °/₀	5	5·0 mins.
·005	6, 4, 6	5·3
·01	6, 8, 5	6·3
·02	10, 4, 9, 5, 11	7·8
·04	25, 20, 12, 9, 16, 14, 23, 26, 9	17·1

The depressant action of HCN on the vitality of the heart is also shown by the fact that after poisoning with ·04 °/₀ HCN the final contraction height of the heart on recovery was on an average 10 °/₀ less than the initial height: after ·02 °/₀ of HCN it was 4 °/₀ less: after ·01 °/₀ HCN it was 8 °/₀ less, and after ·005 °/₀ or less of HCN, it was 3 °/₀ less.

The Action of Sodium Fluoride.

The sodium fluoride solution used was very carefully neutralised, and rendered amphoteric to litmus. On its addition to the oxygenated Ringer's solution a faint cloud of CaF_2 was produced, but no actual precipitate was formed.

The action of sodium fluoride upon the tortoise heart is a complex one, in some of its features difficult to interpret. Part of the most complete series of experiments made is reproduced in Fig. 7. The ·01 °/₀ fluoride solution depressed the contraction height of the heart but slightly, and constancy of contraction was attained in about 24 mins. The ·02 °/₀ fluoride solution depressed the contractions to a somewhat lower constancy level in 23 mins., but on poisoning with ·04 °/₀ NaF there was a sudden jump in the effect produced, and the contractions were depressed to 8 °/₀ their initial height in 15 mins. Constancy was not quite attained when the poison was washed out with Ringer's solution, but in the next experiment, in which ·08 °/₀ NaF was used, it was attained

in 8 mins. (see part of the actual record in Fig. 8). In the next experiment, with ·16 % NaF, it was attained in 5 mins. The constancy level, 6 %, in each of these two latter experiments, was probably no lower than it would have been in the ·04 % experiment, if the poisoning had been continued long enough. As in the HCN experiments, therefore, the effect produced was not proportional to the concentration, but all concentrations from ·04 to ·16 % NaF produced practically the same effect

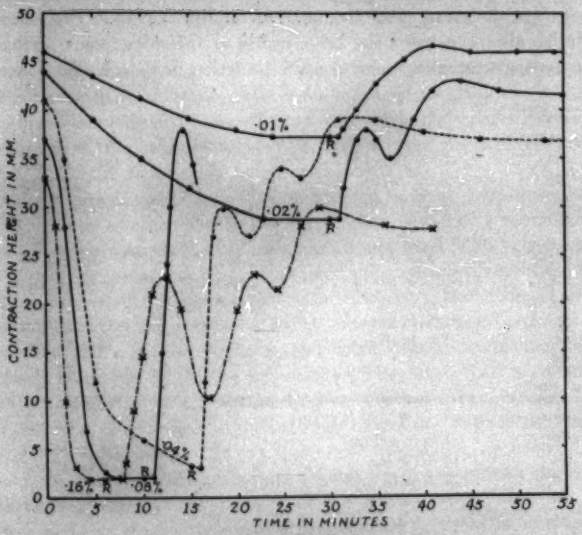

Fig. 7. Sodium fluoride.

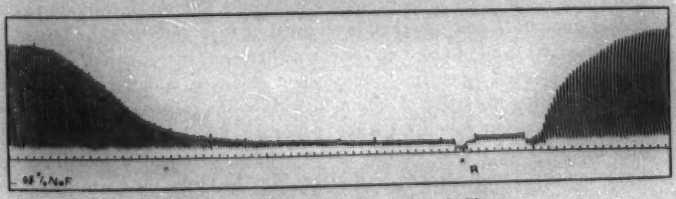

Fig. 8. ×½. Sodium fluoride. Time in 10 secs.

on the heart. The series of poisonings was repeated on the same heart, and the data in the table show that a similar result was obtained. The ·02 % fluoride solution caused more effect than before, but ·04 and ·08 % NaF gave the same constancy level of 7 %. In spite of many attempts, I have never succeeded in confirming this result in its entirety. From the data in the table it will be seen that in most series of experiments ·01 % NaF produced only a slight depressant effect, and ·02 % a moderate one, whilst ·04 % and upwards produced a very marked one; but I never again got the same effect for ·08 % as for ·04 % NaF. The stronger solution always reduced the beats nearly to vanishing point, so that it was no use attempting to investigate the action of ·16 % NaF. It seems

Concentration of NaF	April 1st	April 5th	April 5th (repeated)
·01 %	—	80 % (24 m.)	79 % (15 m.)
·02	66 % (16 m.)	65 % (23 m.)	33 % (26 m.)
·04	5 % (9 m.)	(8 % in 15 m.)	7 % (14 m.)
·08	2 % (7 m.)	6 % (8 m.)	7 % (5 m.)
·16	—	6 % (5 m.)	—

Concentration of NaF	April 21st	June 23rd	July 23rd
·01 %	(70 % in 30 m.)	68 % (10 m.)	96 % (19 m.)
·02	83 % (15 m.)	(9 % in 30 m.)	82 % (22 m.)
·04	(13 % in 20 m.)	1·5 % (30 m.)	(19 % in 30 m.)
·08	(1 % in 10 m.)	—	(2 % in 5 m.)
·16	—	—	—

Concentration of NaF	Aug. 6th	Aug. 23rd	Means
·01 %	—	—	81 % (17 m.)
·02	(84 % in 30 m.)	68 % (11 m.)	66 % (19 m.)
·04	10 % (26 m.)	17 % (33 m.)	8 % (19 m.)
·08	(3 % in 15 m.)	(1 % in 5 m.)	5 % (7 m.)
·16	—	—	6 % (5 m.)

to me probable, however, that sodium fluoride resembles hydrocyanic acid in possessing a range of concentration over which its action on the heart does not produce proportionate effects, only this range, varying as it does from ·04 % and upwards, is so great that it depresses the beats of most hearts nearly to zero, and so can no longer be investigated. This view is supported by entirely independent evidence, which I obtained when measuring the action of sodium fluoride on the gaseous metabolism of the mammalian kidney[1]. After poisoning for 30 mins., I found that the respiration was equally depressed (*i.e.* to about a fourth its normal amount) whether ·1 % NaF or 1 % NaF had been used, whilst the

[1] Vernon. This *Journal*, xxxix. p. 164. 1909.

subsequent recovery of respiration on washing out the NaF with Ringer's solution was equally perfect in both cases.

To return to the contents of the table, the mean values given in the last column afford striking support to the hypothesis of disproportionate action. However, there is one possible source of error which has to be borne in mind. In most of these experiments the fresh heart was first poisoned with the weakest NaF, and then with the progressively stronger solutions, and it might be thought that the sudden jump in the action of the ·04 % NaF was due to depression of the heart's vitality by the previous poisonings. The experiments of April 5th contradict this supposition, and those made on July 23rd do so still further. Thus after the completion on the latter date of the experiments given in the table, the heart was poisoned a second time with ·02 % NaF, and a constancy level of 87 % was attained in 10 mins. It was then poisoned again with ·04 % NaF, the contraction height being reduced to 4 % in 30 mins. Finally it was poisoned a third time with ·02 % NaF, a constancy level of 65 % being attained in 10 mins. Again, on April 21st a repetition of the poisoning with ·02 % NaF gave a constancy level of 76 % in 8 mins.

From the average times of attainment of constancy given in the last column of the table, it appears that with ·01 to ·04 % NaF about 18 mins. were required, whilst with ·08 % NaF there was a sudden drop to 7 mins. Probably this result is erroneous, and dependent on insufficient data. Thus in three out of the five experiments with ·04 % NaF the average time was 12 mins., or a time intermediate between those obtained with ·02 and ·08 % NaF. One would naturally expect the times to be shorter with the greater concentrations than with the smaller, for presumably the amount of NaF which becomes bound up in the cardiac tissues in the former case is less, relative to the concentration, than in the latter, and so a shorter time would be required for it to be abstracted from the perfusing fluid, and for equilibrium to be attained.

On washing out the sodium fluoride from the cardiac muscle with Ringer's solution, the heart does not recover its contractile power at an even rate as on recovery from other poisons, but as a rule shows huge waves, which are probably due to oscillations of tonus. Thus in Fig. 7 it will be seen that during recovery from ·02 % NaF the heart showed one sharply defined wave, followed by a larger and fainter one. On recovery from ·04 % NaF the heart showed three moderate waves, whilst on recovery from ·16 % NaF the heart showed one very large wave, and two smaller ones. For the sake of clearness most of the recovery curve in the ·08 % NaF experiment has been omitted, but it

had three waves on it like the other two curves. A slow drum record of a heart poisoned for 30 mins. with ·04 % NaF is reproduced in Fig. 9. Constancy of contraction height was attained in about 26 mins., and 4 mins. later, when oxygenated Ringer's solution was substituted, the cardiac contractions rapidly increased nearly to their initial value, and

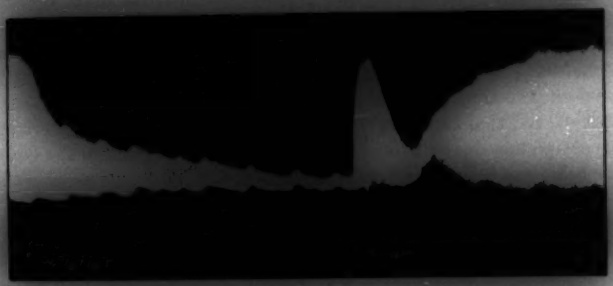

Fig. 9. × ½. Sodium fluoride. Time in mins.

almost as rapidly diminished again, previous to the final and slower recovery. This single wave type of recovery curve is much more common than the type shown in Fig. 7, but no two curves are quite alike.

The production of waves is dependent on the concentration of the NaF, for there were no waves on any of the recovery curves of the 12 experiments made with smaller concentrations of NaF than ·01 %, whilst only one of the 7 experiments made with ·01 % NaF showed a wave. Nine out of the 15 experiments made with ·02 % NaF showed waves of various degrees, whilst all but one of the 31 experiments made with ·04 % or more of NaF showed them. Fig. 10 gives various types of curve, which help to explain the causation of the waves. The three uppermost records were obtained with different hearts, all after 30 minutes' poisoning with ·02 % NaF. The lowest of the three is the commonest in type, and shows that on washing out the NaF with Ringer's solution the heart began to recover, but after a minute or two the recovery was cut short by a more powerful depressant influence. This lasted for five minutes, when the final recovery set in. The uppermost record of the three shows a depressant influence which at first was strong enough to overpower completely the tendency of the heart to recover. The middle record of the three shows the depressant

influence in its most striking form, as it was powerful enough to reduce the beats nearly to zero for a minute or two, after which the heart rapidly and completely recovered.

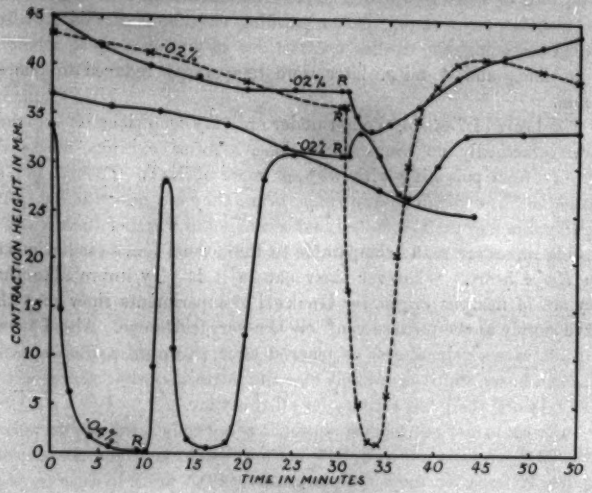

Fig. 10. Sodium fluoride.

As already stated, these waves are probably due to oscillations of tonus, and we have seen that they are provoked, not *during* the NaF poisoning, but at the time when the NaF is being torn away from its combination with the heart substance by the fresh Ringer's solution. Rosenzweig[1] suggested that the oscillations of tonus of the auricular tissue of the *Emys* heart are due to the presence of involuntary muscle fibres and Bottazzi[2] has adduced much evidence in support of this view. Fano[3] states that the ventricle of *Emys* hardly ever gives tonus oscillations, but I find that they are always present to a greater or less degree, and are sometimes very marked. Supposing that they are likewise dependent on involuntary muscle, one must assume that the rupture of the bond between the muscle fibres and the

[1] Rosenzweig. *Arch. f.* (*Anat. u.*) *Physiol.* Suppl. p. 192. 1903.
[2] Bottazzi. *Zeit. f. allgem. Physiol.* VI. p. 104.
[3] Fano. *Beitr. zur Physiol. C. Ludwig gewidmet*, p. 287. 1887.

sodium fluoride excites them to contract, and this contraction prevents the normal beat of the ordinary cardiac muscle fibres. So powerful is the contraction that it may temporarily reduce the beats from a height of 30 mm. or more down to 2 mm. The fourth curve in Fig. 10 is a case in point, for it shows that on washing out the NaF (·04 %) after 9 mins. poisoning, the cardiac contractions at first shot up to a height of 28 mm., and 4 mins. later were temporarily reduced to one of 6 mm.

The heart of *Testudo*, though under ordinary conditions its ventricle shows practically no tonus oscillations, likewise exhibits waves on recovery from poisoning with ·04 % or more of NaF. Though not so marked as those given by the *Emys* heart, they are nevertheless quite considerable, and their existence makes one doubt whether these sodium fluoride waves are really comparable to the normal tonus oscillations of the *Emys* heart. Whatever their nature it is very improbable that they are of nervous origin, for Gaskell's[1] experiments show that the nerve supply to the tortoise ventricle is a very feeble one. Also I found that the waves were almost as marked after 12 hours' perfusion as in the fresh heart, and it is unlikely that the intrinsic cardiac nerves would have retained their full activity for all this time.

In order to get the biggest waves, it is not only necessary to poison with ·03 % or more of NaF, but—as a rule—to continue the poisoning for 30 mins. or more. Thus neither a 15 mins. poisoning with ·04 % NaF, nor a 5 mins. poisoning with ·08 % NaF, produced half as big a wave as a 30 mins. poisoning with ·03 % NaF. Biggest of all was a wave produced by 40 mins. poisoning with ·04 % NaF.

The initial rate of recovery of the heart after poisoning with NaF is very much more rapid than that observed with the four poisons previously described. From the data in the table it will be seen that for hearts poisoned with ·08 and ·04 % NaF the 20 to 80 % recovery

Concentration of NaF	Time of recovery of 20 to 80 % contraction height	Mean
·04 %	1·6, 1·0, 1·3, ·9, 2·0, 1·3, 1·0, ·9, 1·2	1·2 mins.
·05	2·2, 2·5, 2·2, 2·4	2·3
·08	1·6, 1·4, 1·3, 1·1, ·5, 1·4, 1·2	1·2

time averages 1·2 mins. The apparently longer time required after poisoning with ·05 % NaF is probably not a genuine result, as all four experiments were made on two hearts, at a time of the year when their vitality is lowest.

[1] Gaskell. This *Journal*, IV. p. 43. 1883; and VIII. p. 404. 1887.

On washing out the NaF from the heart with Ringer's solution, it approximately recovers its initial height, and shows neither temporary exaltation nor depression. Thus the average final heights differed from the initial ones by +3, +2, −4, −3, and +3 %, after poisoning with ·01, ·02, ·04, ·05, and ·08 % NaF respectively.

The Action of Formaldehyde.

Formaldehyde resembles the other poisons investigated in reducing the heart beats to a constancy level. Probably the reduction is proportional to the concentration, but it is impossible to determine it for certain as not more than one satisfactory experiment can be made on each heart. The time required for the attainment of constancy is

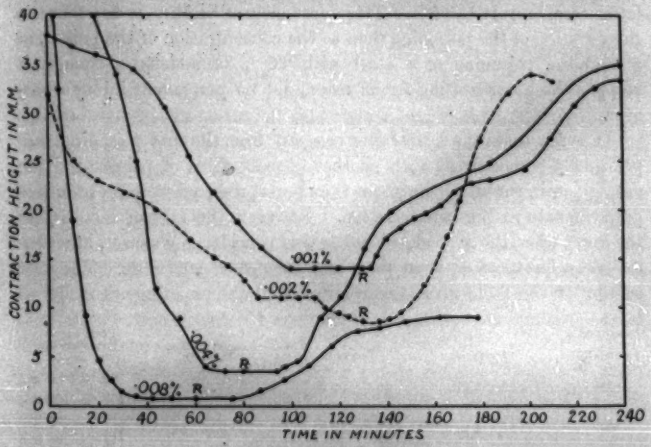

Fig. 11. Formaldehyde.

generally about 80 mins., and this prolonged poisoning permanently injures the heart. Fig. 11 shows four results, all obtained with fresh hearts at the same time of the year (the latter half of July), and so fairly comparable. The ·001 % formaldehyde solution took 100 mins. to reduce the heart to constancy level, and this level was maintained for the remaining 30 mins. of the poisoning. The ·002 % formaldehyde reduced the beats to constancy in 86 mins., and they continued unchanged for 24 mins., but then they began to diminish again. The

cause of this second diminution is unknown, as it does not seem to have been due to the poison reducing the vitality of the heart. Thus the rate of recovery of this heart on perfusion with Ringer's solution was greater than that observed with the other hearts, and the heart more than recovered its initial contraction height.

On poisoning with ·004 % formaldehyde, constancy was attained in 72 mins., and lasted for the remaining 8 mins. for which the poisoning was continued, but this is scarcely long enough an interval to be quite convincing. The experiment with ·008 % formaldehyde is better, as constancy was attained in 42 mins., and persisted for the remaining 18 mins. of the poisoning. However, in both these experiments the poisoning was prolonged enough to injure the heart, as in the former case it recovered only 61 % of its initial contraction height, and in the latter case, only 16 % of it. This depression of vitality is due more to the duration of the poisoning than to the concentration of the poison, as a 15 mins. poisoning of a heart with ·05 % formaldehyde, though it stopped the heart beating for 53 mins., did no permanent injury whatever.

In a few cases the heart, after recovery from the first poisoning, was poisoned a second time with another concentration of poison, and the data given in the table show that the effects produced were more or less proportionate to the concentration. However, the rate of recovery of the heart after the second poisoning was considerably slower, and considerably less perfect, than that after the first poisoning. The last column of the table gives the results of all the experiments made on

Concentration of formaldehyde	April 23rd	July 15th	July 16th	Fresh hearts from July 15th to Aug. 1st
·001 %	42 % (46 m.)	50 % (80 m.)	—	50 % (80 m.) 37 % (100 m.)
·002	(14 % in 45 m.)	27 % (52 m.)	10 % (67 m.)	34 % (86 m.) 19 % (86 m.)
·004	—	—	6 % (72 m.)	6 % (72 m.)
·008	—	—	—	1·4 % (42 m.)

fresh hearts between July 15th and Aug. 1st, and if means be taken of the pairs of results obtained with ·001 % and ·002 % formaldehyde, the average reduction of contraction height induced varies regularly with the concentration.

As can be seen from the curves in Fig. 11, the rate of recovery of hearts poisoned with formaldehyde is very much slower than that observed with other poisons. Instead of the 1·4 min. required after

poisoning with NaF, the 5 to 17 mins. after HCN, and the 5 or 6 mins. after alcohol, chloroform and ether, the heart poisoned with formaldehyde required 24 mins. as a minimum to recover 20 to 80 % of its final contraction height, and in some experiments it required 153 mins. or more. The actual times required by fresh hearts and by hearts after one or more poisonings with formaldehyde are given in the table. The figures in brackets indicate the duration of the poisoning in minutes. The data obtained with fresh hearts seem to show that the rate of

Concentration of formaldehyde	Time of recovery of 20 to 80 % contraction height	
	Fresh hearts	Poisoned hearts
·002 %	34 (50), 43 (90)	24 (16), 53 (45), 53 (60), 151 (90)
·004	104 (80)	—
·008	30 (60)	—
·01	24 (15)	49 (15), 59 (10)
·02	26 (10)	73 (5), 81 (10)
·05	90 (15)	105 (26), 124 (10), 143 (10), 153 (15)

recovery is very irregular, and not dependent on concentration. Probably this deduction is incorrect, as there are several reasons for the irregularities. As a rule, the rate of recovery is slower and slower the greater the concentration of the poison, and the longer the duration of the poisoning, and this is why the time of recovery from ·004 % formaldehyde was greater than that from ·002 %. The shorter time observed in the ·008 % formaldehyde experiment is due to the fact that the final contraction height of the heart was only 16 % its initial height, instead of 61 % or more as in all the other experiments. The quick recovery of the hearts poisoned with ·01 and ·02 % formaldehyde is due to the brief duration of the poisoning.

Latent Period. On washing out a poison from the heart with fresh Ringer's solution, recovery usually begins in a minute or two. As already mentioned, about ·5 to 1 minute of the delay is instrumental in origin, and as can be seen from the data in the table, more than a third of all the experimental results obtained had a latent period of a minute or less; *i.e.* had no genuine latent period at all. In the case of alcohol, ether, HCN and NaF it will be seen that the latent period was not often

Latent period in mins.	Alcohol	Ether	Chloroform	HCN	NaF
1 or less	44 %	25 %	28 %	32 %	39 %
1·1 to 2	34	53	26	44	44
2·1 to 5	17	18	31	24	14
Over 5	5	4	15	0	3
No. of exps.	41	45	39	50	59

over 2 mins., and very seldom over 5 mins. Chloroform, however, by reason of its depressant influence, delayed the recovery of the heart in a considerable proportion of the experiments, and in 15 % of them the latent period was over 5 mins.

Formaldehyde is remarkable for its invariably long latent period, for the accompanying table shows that even with fresh hearts and the minimum concentration of poison it was never less than 3 mins. With greater concentrations it might be 15 mins., or in hearts poisoned a

Concentration of formaldehyde	Fresh hearts	Poisoned hearts
·001 %	3, 3, 8	—
·002	3, 3, 7, 9	3, 7, 10, 19, 27, 36
·004	14	—
·008	15	—
·01	7	9, 12
·02	11, (90)	13, 16, 30, 80
·05	(53)	38, 70, (74)

second or third time with formaldehyde, as long as 80 mins. In a few experiments the beats were reduced to zero by the formaldehyde, and the figures given in brackets denote the times for which the heart beat was completely stopped.

The heart generally reacted to other poisons as it did to formaldehyde, in having the shortest latent period after minimal concentrations of the poison, and the longest after maximal. Again, a heart which had been perfused for a long time, and had been poisoned several times, had a longer latent period than a fresh heart.

The Action of Poisons on the Rate of Heart Beat.

So far the influence of the poisons upon the amplitude of the cardiac contraction has alone been described, though as a rule there is some effect upon the rate of heart beat as well. The slowing is often considerable, and as a heart can relax more completely when beating slowly than when beating fast, the amplitude of contraction is to some extent influenced by the rate. A great amount of labour was expended in measuring and recording the rate of beat during and after the poisoning, but the results obtained were disappointing, as they were individually irregular, though the average results admit of some generalisation. Small differences of effect are of no significance, as a heart, if poisoned repeatedly under similar conditions of time and concentration, is not as a rule affected the same way on each occasion. For

convenience all the results have been classified under four headings. If the beat of the heart was reduced in rate by less than a fourth, the effect is recorded as "nil." If it was stopped completely at any time during or after the poisoning, the result is included in the "stop" class, whilst the intermediate results are split into the two classes given in the table.

Taking first the experiments with alcohol, ether and chloroform, it will be seen that the average effects produced bear a moderate resemblance. Of the chloroform results, about a quarter fall into each of the four classes, whilst of the ether results a quarter similarly fall into each of the first two classes, but the greater concentrations of the drug tend

Reduction in rate of heart beat	Alcohol	Ether	Chloroform	Hydrocyanic acid	Sodium fluoride	Formaldehyde
Nil	18 %	24 %	24 %	51 %	41 %	55 %
¼ to ½	16	29	27	10	24	28
½ or less	27	16	24	23	25	3
Stop	39	31	25	16	10	14
Number of exps.	44	45	37	49	59	29

rather to stop the heart altogether than to slow it, so the "stop" class contains twice as many results as the "half or less" class. Alcohol tends to stop the heart even more than ether, as the beat was arrested in 39 % of all the experiments made, and was greatly reduced in 27 % more of them.

The other group of poisons has as a rule a very different action, for in about half of the experiments made with each of them the rate of heart beat was not reduced at all. Similarly with each of them it was comparatively rare for the heart to be stopped altogether. Evidently, therefore, there is a genuine difference in the average effect produced by a poison, according as it belongs to one class or the other, but it is impossible to predict with any certainty what will be the result in any individual experiment. If the influence of a poison on the rate of heart beat be considered a diagnostic sign of value, the only way to investigate it is to make a large number of observations on different hearts with different concentrations, and take mean results.

As might be expected, the more marked effects upon rate of heart beat were generally brought about by the greater concentrations of poison, and the less marked effects by the smaller ones, but the exceptions were numerous. To demonstrate the effects of concentration, some of the data have been re-classified in the accompanying table. It will be seen that 4 %, or more of alcohol generally stopped the heart, whilst 2 %, or less stopped it but seldom, though it generally slowed

it. The less concentrated ether solutions did not affect the heart at all in half the experiments made, but they nevertheless stopped the heart in a quarter of the experiments. The more concentrated solutions almost always slowed the heart, and they stopped it in 38 % of the

	Alcohol		Ether		Hydrocyanic acid		Sodium fluoride	
Reduction of heart beat	2 % or less	4 % or more	1 in 340 or less	1 in 120 or more	·01 % or less	·02 % or more	·02 % or less	·04 % or more
Nil	29 %	5 %	47 %	4 %	63 %	36 %	50 %	30 %
½ or less	58	25	29	58	33	32	34	66
Stop	13	70	24	38	4	32	16	4
No. of exps.	24	20	21	24	27	22	32	27

experiments. The less concentrated HCN solutions as a rule did not affect the heart at all, and they scarcely ever stopped it, whilst even the more concentrated solutions did not affect it in 36 % of the experiments. The less concentrated NaF solutions did not affect the heart at all in half the experiments, whilst the more concentrated solutions did not affect it in a third of the experiments. The more frequent stoppage of the heart by the less concentrated NaF was due to the abnormally feeble condition of two of the hearts subjected to these solutions. These hearts will be referred to again below.

The Action of Poisons on Hearts depressed in Vitality.

Mention has been made from time to time of concentrations of poison sufficient to injure the heart and lower its vitality, but so far as possible all experiments on such injured hearts have been omitted from the records given. The reason of this is that a heart, once lowered in vitality by too much poison, or by prolonged perfusion, is no longer the same organ, and reacts differently on subsequent poisoning. Such a difference of reaction was observed with all the poisons investigated, with the doubtful exception of HCN, but especially with chloroform, so the chloroform results will be described first.

Chloroform. The experiment previously described affords striking evidence of altered reaction, but a more complete one is shown in Fig. 12. The fresh heart was poisoned for 30 mins. with 1 in 8000 chloroform, and the contraction height was depressed to a constancy level of 67 %, the initial height in 11 mins., and showed no further depression during the remaining 19 mins. of poisoning. After being poisoned with 1 in 4000 chloroform, the heart was poisoned a second time with the 1 in 8000 solution. The beats were thereby depressed

to 40 % their initial height. A few hours later, after the heart had meanwhile been poisoned twice with dilute chloroform solutions, the experiment was repeated again, and 3¼ hours later still it was repeated for the third time. In each experiment the beats of the heart were rapidly reduced to constancy level, but the depression was more and more marked the lower the vitality of the heart. Also, as can be seen from the slope of the curves, the rate of recovery was somewhat slower in the third poisoning than in the second, and in the fourth than in

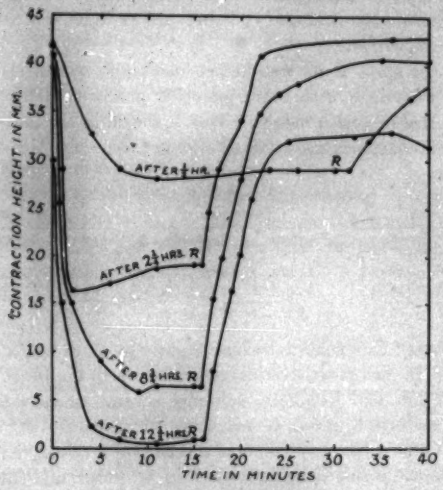

Fig. 12. Chloroform.

the third. It was slowest of all in the first poisoning, probably because the chloroform saline was perfused twice as long as in the subsequent experiments. A more remarkable instance of such delayed recovery was observed in a heart which was perfused continuously for 2½ days. The time of recovery of 20 to 80 % of the contraction height of the heart, instead of the 6 mins. required by fresh hearts, was 10 mins. after 19½ hours' perfusion, 25 mins. after 31 hours' perfusion, and 140 mins. after 50 hours' perfusion.

This increasingly depressant effect of successive doses of chloroform was noted by Sherrington and Sowton in their experiments on the

cat's heart. They found that 3 min. poisonings with 1 in 20,000 chloroform reduced the contraction height to 36, 39, 25, 31, 13, and 11 % respectively in successive experiments. I have myself observed it invariably, whatever the strength of chloroform used. Thus on poisoning with a 1 in 16,000 solution after ½, 7, and 10 hours' perfusion, the contraction height was reduced to 79, 38, and 22 % respectively. In another experiment with the same concentration it was reduced to 68 and 46 % after 1½ and 5½ hrs. respectively. In still another, to 53 and 2 % after 5 and 24 hrs. respectively. On poisoning with 1 in 4000 chloroform after 3, 5, and 12 hours' perfusion, it was reduced to 4, 2, and 1 % respectively.

Ether. In spite of its well-known excitatory action on the heart, successive poisonings with ether generally produce rapidly increasing depression of contraction height. This is shown in the following data. In all of them constancy was attained after the respective times indicated in brackets.

1 in 120 ether		1 in 240 ether		1 in 960 ether	
Perfusion time	Contraction height	Perfusion time	Contraction height	Perfusion time	Contraction height
2 hrs.	48 % (8 m.)	1½ hrs.	53 % (4 m.)	½ hr.	80 % (10 m.)
8 hrs.	26 % (12 m.)	5½ hrs.	34 % (11 m.)	8½ hrs.	25 % (15 m.)
10½ hrs.	2 % (5 m.)	11½ hrs.	30 % (13 m.)		

The recovery time tends to become slightly prolonged after successive poisonings. In the most striking result obtained, the heart had been perfused 31 hrs. and had been poisoned several times with alcohol, chloroform and HCN before it was poisoned with 1 in 60 ether, and yet the 20 to 80 % recovery time was the normal 6 mins. After 32 hrs. perfusion it was poisoned again with 1 in 60 ether, and the recovery time was increased to 9 mins., whilst a third poisoning after 48 hrs. perfusion gave a recovery time of 19 mins.

Alcohol. Repeated poisonings of the heart with alcohol do not seem to do it much harm, and often the depression of contraction height produced by a second poisoning is no greater than that produced by the first. Two series of experiments illustrating this fact are recorded in a previous table. Though the second series of poisonings was in each case made about 7 hrs. after the first one, the average depression of contraction height was only 2 % greater. In another series, however, in which the first poisonings with 1, 2, and 4 % alcohol depressed the contraction height to 76, 52, and 4 % respectively, a repetition of the experiments about 3½ hrs. later gave depressions of 57, 12, and 2 %

respectively. In another experiment the heart was poisoned with 4 % alcohol after 1½ hrs. perfusion, and had its beat reduced to 13 %, whilst after 24 hrs. perfusion it was reduced to 5 %.

The rate of recovery of the heart from alcohol is not much influenced unless perfusion be continued for a day or more. The most striking result was given by a heart poisoned with 8 % alcohol. After 3 hrs. perfusion, its 20 to 80 % recovery time was 4 mins.: after 20 hrs. perfusion, 9 mins., and after 45 hrs. perfusion, 29 mins. Another heart, poisoned with 4 % alcohol after 1½ hrs. perfusion, had a recovery time of 6 mins., and after 24 hrs. perfusion, one of 9 mins. Still another heart, poisoned with 2 % alcohol after 24 hrs. perfusion, had a recovery time of 11 mins.

Sodium fluoride. If a heart be subjected to this poison a second time within 5 hrs., it does not as a rule suffer any further depression of contraction height. This point is brought out by the four sets of experiments mentioned in a previous section.

If a longer interval elapses before a poisoning is repeated, the depression of contraction height produced on the second occasion is generally much greater, as can be seen from the following data:

·005 % NaF		·04 % NaF		·04 % NaF		·04 % NaF	
Perfusion time	Contraction height	Perfusion time	Contraction height	Perfusion time	Contraction height	Perfusion time	Contraction height
8 hrs.	28 % (21 m.)	2½ hrs.	29 % (15 m.)	¾ hr.	17 % (33 m.)	2¾ hrs.	(13 % in 20 m.)
24 hrs.	(9 % in 5 m.)	8½ hrs.	(4 % in 20 m.)	7¾ hrs.	1 % (11 m.)	9 hrs.	(1 % in 15 m.)

The recovery time is distinctly increased on prolonged perfusion. Thus one heart, poisoned with ·1 % NaF, had a 20 to 80 % recovery time of 1·2 mins. after 8½ hrs. perfusion, one of 2·1 mins. after 12 hrs. perfusion, and one of 13 mins. after 29 hrs. perfusion. Another heart had recovery times of 1·6 and 4·1 mins. on poisoning with ·04 % NaF after perfusion for 2¼ and 8 hrs. respectively, whilst the same heart had recovery times of 1·4 and 2·5 mins. when poisoned with ·08 % NaF after perfusion for 3 and 9 hrs. respectively. In three other hearts the increase of recovery time on poisoning a second time after 4 to 6½ hrs. interval was only ·3 or ·4 min.

Formaldehyde. Only one complete experiment was made with this poison, for the slowness of its action makes them very prolonged. On poisoning a heart with ·002 % formaldehyde after ¼ hr. perfusion, constancy level of 19 % was attained in 86 mins. On repeating the poisoning after 4½ hrs. perfusion, a level of 13 % was attained in 85 mins.

Another repetition after 10½ hrs. perfusion gave a level of 15 %, in 82 mins., whilst a final repetition after 24 hrs. perfusion gave a reduction of contraction height to 4 % in 90 mins. The 20 to 80 % recovery time in the first of these poisonings was 43 mins., and in the second 151 mins. In the third it was much slower still, and in the fourth slowest of all. In fact it was so slow in both these experiments that it was not recorded in its entirety. Another heart, poisoned with ·05 % formaldehyde after ¾ hr. perfusion, had a recovery time of 90 mins.: when poisoned again after 7 hrs. perfusion, one of 105 mins., and when poisoned a third time after 26 hrs. perfusion, one of 143 mins. Still another heart, poisoned with ·02 % formaldehyde after 2 and 4¾ hrs. perfusion, had recovery times of 26 and 73 mins. respectively. Hence hearts not only recover from formaldehyde much more slowly than from any of the other poisons investigated, but the rate of recovery is relatively much more retarded on repeated poisonings.

Hydrocyanic acid. In the set of "repeated" experiments given in a previous table, which were made on an average 9½ hrs. after the first set, the relative depression of contraction height produced by ·0025 to ·01 % HCN was slightly less than during the first poisonings. In two other experiments, in both of which the heart was poisoned with ·04 % HCN for 10 mins., a corresponding effect was observed. Thus in one of them the first poisoning, made after 21 hrs. perfusion, reduced the contraction height to 3 %; whilst the second one, made after 45 hrs. perfusion, reduced it to 16 %. In the other experiment the first poisoning, made after 10 hrs. perfusion, reduced it to 3 %, and the second, made after 26 hrs., to 5 %. In both these experiments, however, the initial contraction height of the heart just before the second poisoning was only about a third as great as it was before the first poisoning. Still these results, so far as they go, disagree with those obtained with other poisons. By an oversight I did not think of repeating them until after my supply of *Emys* was exhausted.

Seasonal variations in vitality. The experiments recorded in earlier sections of this paper show that as a rule the sensitiveness of the heart to poisons does not vary much at different times of the year. Experiments were made in every month of the year but February, May and November, and no appreciable difference of reaction was noticed except in the experiments made in March. The tortoises then used were obtained from a different source from the others, and were said to have been kept in England all the winter, whilst the more vigorous specimens I obtained in April were said to have been freshly imported. Whatever

the reason, the hearts of the March tortoises were very much more sensitive to poisons than any of the others, and especially so to sodium fluoride. Vigorous hearts have their contraction height reduced by ·02 % NaF to a constancy level of 66 % in about 20 mins. on an average, but some of these hearts had their beat stopped by a few minutes' perfusion with ·001 or ·002 % NaF. One heart was perfused for 3 to 10 min. periods at approximately hourly intervals with ·02, ·01, ·005, ·0025, ·00125, ·000625, ·0003125, ·0002, and ·0001 % NaF solutions, and by the first five concentrations the heart was stopped altogether after a few minutes, whilst the remaining four concentrations reduced its beat to 6 % or less of the initial contraction height. On washing out with Ringer's solution, the heart always recovered well, but only after a latent period of 3 or more minutes. Also the 20 to 80 % recovery times, instead of the usual 1 or 2 minutes, were 8, 7, 5, 4, 5, 4, 8, 5, and 5 mins. respectively.

The sensitiveness of these hearts to alcohol was also very marked. Thus the beats of one heart were stopped by 1 % alcohol, whilst ·5 % alcohol reduced them to 5 % their initial height, and ·25 % alcohol, perfused subsequently, to 2 % their height. All concentrations of ether from 1 in 120 to 1 in 960 stopped the hearts beating, though no heart tested at other times of the year was stopped by less than 1 in 120 ether. Also 1 in 8000 chloroform stopped the hearts, though no heart tested at other times was stopped by less than 1 in 4000 chloroform. Even ·01 % HCN stopped the heart in one experiment, though no heart tested at other times was stopped by less than ·04 % HCN. The effect of formaldehyde poisoning was likewise greater than usual, so it follows that hearts are more sensitive to all poisons, not only when their vitality is lowered by prolonged perfusion and by poisoning, but when the tortoises from which they are obtained are in a poor condition. Such a condition is probably due chiefly to lack of nourishment, such as one would expect at the end of the hibernating season.

General Conclusions.

The experimental results above described seem to indicate that of the six poisons investigated, alcohol, ether and chloroform enter into a similar kind of union with the contractile substance of cardiac muscle. The depressant effect on the amplitude of cardiac contraction produced by each of them is proportional to their concentration, or presumably

definite amounts of the poison proportional to the concentration become temporarily bound up in the muscle substance. In that the rate of recovery of the heart on perfusion with fresh Ringer's solution is the same for all three poisons, it is probable that they all break away from their loose combination at the same rate. As to the nature of the union we have no definite information, but there is much to be said for Moore's hypothesis[1], according to which it is dependent on what he terms *molecular valency*. He points out that many cases of combination are known, such as of salts and their water of crystallisation, of colloidal sulphides and H_2S, of dextrose and lecithin, in which the combining molecules are completely saturated in respect of their atomic valencies, but in which exact stoichiometric relationships exist between the numbers of combining molecules. Frequently, also, there is a considerable evolution of heat accompanying the union, and so he considers that molecules may possess a valency, not attached to any constituent atom, but in their entirety, and by means of this valency they can enter into combination with other molecules. When crystalloids enter into such molecular combination with colloids, exact stoichiometric relationships are not as a rule observed, because of the huge size of the colloidal aggregates to which each crystalloidal molecule is united. Also there may be molecular dissociation, or again the whole mass of colloid present may not be the active mass. Of other hypotheses, Meyer and Overton state that narcotics are substances which dissolve in the tissue lipoids, in that their narcotic action is proportional to their partition coefficient lipoid/water. Höber[2], again, suggests that narcosis is due to the excitability of the colloidal lipoids of protoplasm being paralysed by the narcotic which accumulates in them up to a definite concentration.

It is evident that these three hypotheses can readily be combined, and that narcotics such as alcohol, ether and chloroform may be held to exert their action by the combination—by means of molecular valency—of numbers of their molecules proportional to their concentration with certain colloidal constituents of the bioplasm. These colloids may be lipoids only, or more probably, partly lipoids and partly proteins[3], and their lability would thereby for the time being be diminished or completely paralysed. In that different narcotics have distinct differences in their

[1] B. Moore. *Arch. di Fisiol.* VII. p. 519. 1909.

[2] Höber. *Pflüger's Arch.* CXX. p. 492. 1907; *Zeit. f. allgem. Physiol.* X. p. 178. Ref. 1910.

[3] Cf. Moore and Roaf. *Thompson Yates and Johnston Laboratories Reports*, VI. Pt. 1, p. 151. 1905.

course of action (*e.g.* excitatory or depressant), it is probable that they do not all combine in quite the same way, or that they combine to different degrees with two or more different colloidal systems.

Of the three other poisons investigated, there seems little doubt that hydrocyanic acid enters into a definite chemical combination, by means of atomic valencies, with certain constituents of the tissues The amount so combining is not proportional to the concentration of the poison in the perfusion liquid, but tends to attain the same value even though the concentration be varied as 1 to 8. The hydrocyanic acid, when bound up in the cardiac substance, only reduces the beats to about half their normal amplitude, so it does not stop the essential vital processes of heart muscle, whatever it may do when bound up in the central nervous system. I have suggested elsewhere[1] that it enters into combination with the aldehyde groupings to which Loew attributes the lability of living protoplasm, and if this is so it follows that it only combines with some of them, and not with all, as the heart would thereby be completely paralysed. Hydrocyanic acid readily combines with a number of chemical substances, and so there would be no reason for supposing that it combined with aldehydes rather than with other groupings in the tissues were it not that sodium fluoride has a similar affinity for aldehydes. As we have seen, it likewise appears to enter into some sort of chemical combination with certain of the tissue constituents, for its action is probably not proportional to its concentration, and it has in addition the extraordinary power of inducing tonus oscillations when washed out of the heart substance. This property might be taken to show that whatever groupings it combines with in the tissues, they are not the same as those with which HCN unites. It is possible, however, that this extra effect is due to its combining with a greater proportion of the aldehyde groupings than HCN does, for it certainly acts more powerfully in reducing the heart beat. Still other confirmatory evidence in favour of this aldehyde hypothesis is found in the action of acid sodium sulphite. This substance readily combines with aldehydes, and I found that its action upon the gaseous metabolism of the mammalian kidney is similar to that of HCN and NaF.

Formaldehyde, like hydrocyanic acid, in all probability enters into definite chemical combination with certain tissue constituents by means of atomic valencies; perhaps, as Loew suggests, with some of the amino groupings. It will be remembered that in comparison with NaF and

[1] Vernon. This *Journal*, XXXIX. p. 159. 1909.

HCN its rate of combination is very slow, about 80 minutes being required for the attainment of equilibrium instead of the 10 to 20 minutes required by them. Of more import is the very slow rate at which the poisoned heart recovers on perfusion with fresh saline, and the fact that this recovery rate is progressively very much longer after repeated poisonings. Formaldehyde, as a volatile substance of small molecular weight, might be expected to break away from the tissues readily, and as it does not do so, I think it highly probable that once bound up in them it is unable to break away at all, but is got rid of only after an initial process of oxidation. Hence the rate of recovery of the poisoned heart muscle is a measure of its intrinsic oxidising capacity, and is therefore greatly influenced by its vitality.

The difference in the action of formaldehyde from that of HCN and NaF is also shown in its effect on the respiratory quotient. Thus I found that both in the mammalian kidney and in the tortoise heart it temporarily lowered the quotient to ·4 or so, whereas the other two poisons did not affect it.

As the result of the numerous experiments by Waller[1], Veley and Miss Kemp on the toxic effects of various acids, bases, alcohols and salts on the isolated frog's sartorius, Veley[2] concludes that the observed effects are due to chemical changes taking place between the muscle stuff and the compounds. He points out that the temperature coefficients observed were almost the same as those yielded by definite chemical changes, and are of a much higher order than the constants for physical changes. Also the relative reactivities of certain organic acids on muscle generally followed the same order as their relative avidity values, or are dependent on their ionisation. Again, the results obtained with mixed solutions of nicotine with quinine and other bases[3] indicated a subdivision of muscle stuff between the respective pairs of bases precisely as an acid subdivides itself between two bases.

It seems to me that these and other experiments of a somewhat similar nature carried out by A. V. Hill[4] and by numerous other investigators undoubtedly indicate that the interaction of poisons with

[1] Waller. This *Journal*, XXXVII. *Proc.* pp. lxxi and lxxxi; XXXVIII. *Proc.* p. x; *Proc. Roy. Soc.* LXXXI. B, p. 545; *Q. J. Exp. Physiol.* III. p. 97. 1910. Veley and Waller. This *Journal*, XXXIX. *Proc.* pp. xix, xxvii, and xxxvi; XL. *Proc.* p. xviii; *Proc. Roy. Soc.* LXXXIII. B, pp. 147 and 205. Veley. *Proc. Roy. Soc.* LXXXII. B, p. 217. Kemp. This *Journal*, XXXVII. *Proc.* p. xlix.

[2] Veley. *Q. J. Exp. Physiol.* III. p. 233. 1910.

[3] Veley and Waller. *Proc. Roy. Soc.* LXXXII. B, p. 333. 1910.

[4] A. V. Hill. This *Journal*, XXXIX. p. 361. 1909.

protoplasm is a chemical rather than a physical process, but they do not prove that the chemical reactions are all of one and the same character. The evidence adduced by me negatives this, and so it seems better to adopt some such hypothesis as that of molecular unions and atomic unions suggested by Moore.

Summary.

On perfusing the tortoise heart with oxygenated Ringer's solution containing alcohol, the amplitude of contraction is quickly reduced by an amount more or less proportional to the concentration of the alcohol. After about 12 minutes equilibrium is established, and the heart then goes on beating at a constant level for the remainder of the poisoning. On substitution of fresh Ringer's solution it recovers its original contraction height at a rate independent of the concentration of the alcohol the heart was poisoned with. On an average 1 % alcohol reduces the beats to 87 % their initial height: 2 % alcohol, to 60 %, and 4 % alcohol, to 17 %.

Ether acts similarly to alcohol, saline containing 1 of ether in 960 reducing the beats to a constant level of 85 % their initial height on an average: 1 in 480 ether to 75 % their initial height: 1 in 240 ether to 63 %: 1 in 120 ether to 33 %, and 1 in 60 ether to 16 %. Chloroform likewise reduces the cardiac contractions to a constant level by an amount roughly proportional to the concentration. The average times of attainment of constancy on ether and chloroform poisoning were 10 and 12 minutes respectively, whilst the rate of recovery of the poisoned hearts on washing out with fresh saline was the same as after alcohol poisoning, and was similarly unaffected by the concentration of the poison.

Hydrocyanic acid tends to reduce the cardiac contractions to a constant level in 15 or more minutes, but the effect produced is *not* proportional to the concentration. With concentrations of ·000625 % HCN or less it is proportional, but concentrations of ·00125 % up to ·01 % may produce almost the same effect, or presumably the same amount of the poison tends to become bound up in the cardiac substance in spite of the variations in concentration. Greater concentrations than ·01 % HCN reduce the beats still further, but they permanently injure the heart.

Sodium fluoride reduces the beats to a constant level in 7 to 19 minutes, but probably the effect is not proportional to the concentration,

for ·01 % NaF reduces them to 81 % their initial height, and ·02 % NaF to 66 % their height, but ·04 % NaF to 8 % their height, and sometimes ·08 % NaF has no more effect than ·04 % NaF. On washing out the NaF with fresh saline, the ventricle generally shows one to three huge waves of tonus oscillation.

Formaldehyde very slowly reduces the contractions to a constant level proportional to the concentration (·001 % to ·008 %), and on washing out with fresh saline the heart very slowly recovers after a long latent period.

Alcohol, ether and chloroform act similarly on the rate of heart beat, as each of them stopped the heart in a fourth to a third of all the experiments, and slowed it in about half of them. Formaldehyde, HCN and NaF likewise act similarly in that they very seldom stopped the heart absolutely, and in half of the experiments had no effect on it whatever.

Hearts depressed in vitality by prolonged perfusion or by much poisoning are thereby rendered more sensitive to the action of poisons, and especially so to chloroform. Thus on poisoning a heart with 1 in 8000 chloroform saline after it had been perfused for ¼, 2¼, 8¼, and 12½ hours, the contraction height was reduced in the successive experiments to a constant level of 67, 40, 16, and 3 %. Hearts do not become much more sensitive to alcohol or to NaF until they have been perfused for several hours.

It seems probable that alcohol, ether and chloroform enter into a loose combination with the colloidal lipoids or other constituents of the tissues by means of molecular valencies, whilst HCN, NaF and formaldehyde enter into definite chemical combination by means of atomic valencies.

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THE ACTION OF ATROPINE, PILOCARPINE AND PHYSOSTIGMINE.

By ARTHUR R. CUSHNY.

(From the Pharmacological Laboratory, University College, London.)

SCHMIEDEBERG and KOPPE, working on the action of muscarine on the heart, were struck by the absence of effect when atropine had been injected previously, and on the ground that atropine was known to paralyse the vagus they formulated the theory that muscarine stimulates this nerve. Subsequently pilocarpine proved to resemble muscarine so closely in its action that it has generally been described as of the same nature. The exact point of action of these alkaloids on the path of the nerve impulse has been located differently by different workers, but the more recent supporters of this view are unanimous in placing it in the "terminations" of the post-ganglionic fibres. The later investigations of the behaviour of the heart under these alkaloids have only confirmed more exactly the resemblance between it and that under stimulation of the inhibitory fibres[1]. The action on the iris and on the salivary and sweat glands also resembles that of stimulation of the motor and secretory fibres, and the antagonism of atropine is so complete that here also the action on the terminations of the post-ganglionic fibres has been generally accepted. It is true that Anderson has shown that pilocarpine continues to contract the pupil after degeneration of the post-ganglionic fibres of the motor oculi, but this merely pushes the seat of action further towards the periphery, indicating that it affects not the anatomical terminations of the nerve fibres but some receptive substance (Langley) interpolated between them and the final contractile substance. The receptive substances for pilocarpine, muscarine and atropine have thus been localised in the extremities of the path of nerve impulse on the same grounds as have applied in the cases of adrenaline, nicotine and curarine.

[1] See MacLean, *Biochem. Journ.* III. p. 1. 1907; and IV. p. 66. 1909.

The active movements of the intestine and other abdominal organs under muscarine and pilocarpine and the antagonism of atropine suggested a similar mechanism here, and it was generally assumed that pilocarpine stimulated and atropine paralysed the terminations of the motor nerves of the intestine. Some discrepancies had been noted, but this view was first discredited by Bayliss and Starling[1], who showed that while atropine antagonises the effects of pilocarpine on the bowel as completely as on the heart and iris, it does not prevent the effect on the bowel of stimulation of the motor and inhibitory nerves. Some doubt has been expressed by Fröhlich and Loewi[2] as to the correctness of this interpretation of Starling and Bayliss' experiments, and I may therefore state that soon after the appearance of their paper, their results with atropine were confirmed in a series of experiments performed in my laboratory by Ide and Torrey. From these unpublished experiments I select a tracing in which the movement of the dog's small intestine was recorded by means of a balloon inserted in the lumen and connected with a piston recorder (Fig. 1). The reaction to stimulation of the left vagus is shown in *A* before, and in *B* after 2 mg. of atropine. The effect of stimulation is practically identical while pilocarpine injected afterwards had no result. The reflex reaction to mechanical stimulation of the bowel by pinching was unchanged by atropine. These results, in confirmation of Bayliss and Starling, indicate that the violent movement of the intestine induced by pilocarpine may be antagonised by atropine without interruption of the paths of nerve impulse to the bowel.

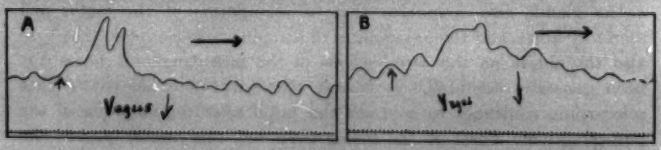


Fig. 1.

Similar observations have been made in regard to other organs. Thus De Zilwa[3] found muscarine cause a marked increase in the tone of the retractor penis, which was abolished by atropine, yet the muscle continued to contract on nerve stimulation. The violent contractions

[1] This *Journal*, XXVI. p. 25. 1901.
[2] *Arch. f. exp. Path. u. Pharm.* LIX. p. 84. 1908.
[3] This *Journal*, XXVII. p. 200. 1901.

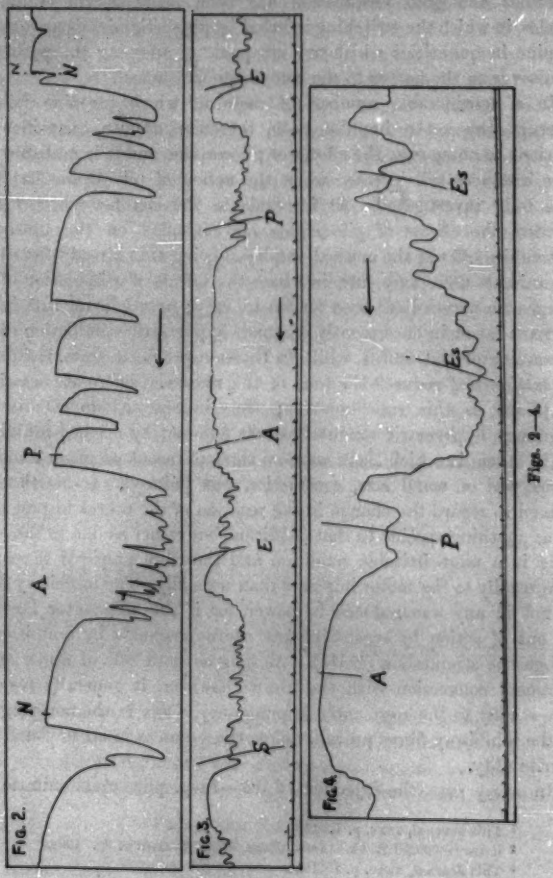
of the rabbit's uterus under pilocarpine are arrested by atropine though stimulation of the hypogastric nerves continues to be effective[1]. Prof. Langley informs me that he has recently observed a similar effect in the bladder, atropine preventing the pilocarpine contraction but not preventing that due to stimulation of the pelvic nerves in the cat. A somewhat analogous observation has been made in the voluntary muscles, in which the twitching induced by physostigmine is arrested by atropine in quantities which are incapable of affecting the passage of impulses from the nerves to the contractile substance[2].

In a considerable number of cases in which atropine fails to interrupt the nerve impulse path, therefore minute quantities are sufficient to antagonise the effects of pilocarpine, and it is probable that these instances will increase when the action of pilocarpine has been more fully investigated. At intervals in the last few years, I have recorded the effects of pilocarpine and atropine on the uterus as opportunity offered, the method employed being that already described[3]. The animals used were cats, in which the effects of stimulation of the hypogastric nerves have been shown to vary; as a general rule in the pregnant cat or in one recently pregnant hypogastric stimulation causes contraction of the uterus, while in the non-pregnant state it inhibits contraction and reduces the tone of the muscle. There are occasional exceptions to this rule however; thus in one or two cats in late pregnancy, hypogastric stimulation was followed by marked inhibition, and in others in which there were no signs of recent pregnancy and the uterus was of small size, contraction was induced. I am therefore inclined to regard the change in the reaction of the uterus in pregnancy not as anything specific to that condition, but rather as due to the organ being in a more irritable condition and therefore prepared to respond more readily to the motor impulses than normally. The inhibitory fibres are not in any way reduced in power, for if the augmentor fibres be put out of action by ergotoxine the uterus responds by inhibition on hypogastric stimulation (Dale). As long as both sets of fibres are in functional connection with the uterus however, it generally responds more readily to the augmentor in pregnancy, while in the non-pregnant cat the inhibitory fibres prevail, unless the organ is in an unusual state of irritability.

In every case the injection of 0·5—1 mg. pilocarpine nitrate was

[1] This *Journal*, XXXV. p. 1. 1906.
[2] Edmunds and Both. *Amer. Journ. Physiol.* XXIII. p. 28. 1908.
[3] This *Journal*, XXXV. p. 1. 1906.

followed by the same changes as arose from stimulation of the hypogastric. In some experiments in addition to electrical stimulation, nicotine or adrenaline was injected and the movements of the uterus were found to be the same from all three alkaloids. In Fig. 2 the



tracings from the uterus of a pregnant cat are recorded[1]. Electrical stimulation of the hypogastrics had previously caused contraction. Nicotine (N), 1 mg., caused powerful contraction. Pilocarpine (P) also induced powerful prolonged contraction, which was released by 1 mg. atropine (A). After this, nicotine (N) was again followed by contraction of shorter duration, but pilocarpine had no effect even in large doses. Electrical stimulation of the hypogastrics also induced contraction. In Fig. 3, the tracing was obtained from a non-pregnant cat. Hypogastric stimulation (E) induced relaxation, and the same result followed the injection of pilocarpine (P), the uterus beginning to contract feebly after a time, but only returning to its normal activity after atropine (A) had been injected. Hypogastric stimulation again induced relaxation and adrenaline (S) had the same effect, but pilocarpine was now inactive.

In this experiment as in many others, pilocarpine induced inhibition of the uterus exactly resembling the effects of nerve stimulation, while in the pregnant cat from which Fig. 2 was derived its injection was followed by contraction, again resembling the effects of nerve stimulation. In two experiments in which hypogastric stimulation caused a short contraction followed by inhibition, pilocarpine had the same effect.

The action of pilocarpine on the uterus therefore follows exactly that of nerve stimulation and might be ascribed to its affecting some point on the path of the nervous impulse in the same way as the action of nicotine or adrenaline. Against this however is the antagonism of atropine which prevents the action of pilocarpine while permitting that of nerve stimulation, nicotine, or adrenaline. And the difference between pilocarpine and these is not merely a quantitative one, for while 1 mg. of atropine is sufficient to neutralise the effect of 10 mg. pilocarpine, the quantity of adrenaline necessary to cause a uterine contraction is not appreciably altered by this amount of atropine, nor is the minimal strength of current required to stimulate the hypogastrics changed in any way. Adrenaline and pilocarpine thus act in the same way as hypogastric stimulation on the uterus, but pilocarpine differs from the others in its being completely antagonised by small quantities of atropine, which have no effect whatever on the reaction to the others.

Dale has shown that ergotoxine antagonises the motor effect of adrenaline on the uterus, and it was therefore of interest to determine whether it could also antagonise that of pilocarpine. Dr Dale kindly sent me a preparation of ergotoxine phosphate for this purpose.

[1] In all the tracings from the uterus the downward movement indicates contraction, the upward relaxation.

Exp. 1. A cat in the last weeks of pregnancy, anæsthetised with paraldehyde; tracheotomy and carotid cannula for blood-pressure, levers attached to one horn of the uterus in the space between two fœtal sacs.

3.10. Adrenaline injected intravenously caused strong contraction followed by a return to rest after about two minutes.

3.19. Pilocarpine nitrate (0·5 mg.) was followed by a series of rather weak contractions lasting until 3.24 and then by one powerful contraction (spontaneous?) the organ coming to rest at 3.28.

3.31. Pilocarpine nitrate (0·5 mg.) caused a powerful contraction followed by a series of movements in which no complete relaxation was reached. These still continuing.

3.36. A solution of ergotoxine phosphate dissolved by the aid of a trace of sodium hydrate was injected. A strong contraction set in with a series of secondary movements.

3.41. The uterus still in contraction: injection of adrenaline.

3.43. Relaxation of uterus; small weak contractions cause undulations in the tracing, but the stronger movements are inhibited until 3.54 when a slow, strong, fairly regular rhythm sets in.

4.3. Pilocarpine ½ mg. arrests this rhythm, the uterus remaining motionless in complete relaxation for 5½ minutes. The movements then recommence.

4.13. Pilocarpine ½ mg. again arrests these movements.

4.15½. Atropine 1 mg. injected.

4.20. Movements recommence.

Subsequently pilocarpine had no effect.

A similar effect is seen in Fig. 5, *a*, *b*, *c*, obtained from a cat in advanced pregnancy. In Fig. 5 (*a*) a short contraction is seen from a very small dose of adrenaline (A), and prolonged activity from the subsequent injection of 0·5 mg. pilocarpine nitrate (P). A large dose of ergotoxine phosphate (about 2½ mgs.) was now injected. Later, in Fig. 5 (*b*), pilocarpine (P) 0·5 mg., induced inhibition, which was more marked after a second injection of the same amount. Atropine (R) 1 mg. now injected was followed by contractions after a short time and increased tone of the uterus. In Fig. 5 (*c*) the effect of a small dose of adrenaline injected subsequently is shown to be inhibition resembling that of pilocarpine. The change from rest to activity as the prevailing phase during the experiment may have been due to the ergotoxine, but occurs usually during a long experiment.

This experiment was repeated on other animals and gave the same result. The contractor effect of pilocarpine on the pregnant cat's uterus was found to disappear after ergotoxine and to be replaced by an inhibitor action. I was not able to arrest by ergotoxine the movements of the uterus induced by pilocarpine in such a striking way as by atropine, because ergotoxine itself induces contraction in the beginning of its action and often causes a persistent rhythm of the

organ afterwards. But the subsequent injection of pilocarpine, instead of causing contraction as before the ergotoxine, now induced marked inhibition. The effects of pilocarpine on the blood-pressure and pulse rate were not changed by the previous injection of ergotoxine.

Exp. 2. Cat, late in pregnancy, anæsthetised with paraldehyde, levers attached as in Exp. 1. Stimulation of the hypogastric caused powerful contraction of the uterus. The injection of 1·5 mg. ergotoxine phosphate was followed by strong contraction followed by rapid contractions and partial relaxations for six minutes. The blood-pressure fell rapidly and artificial respiration was necessary. Hypogastric stimulation caused a weak contraction followed by inhibition, then on the third stimulation relaxation alone. Subsequent stimulation gave no further contraction, but pure inhibition. Pilocarpine (1 mg.) injected now inhibited the uterus which remained in the relaxed position for 14 minutes. Atropine (1 mg.) was now injected and 2½ minutes later contractions returned, although they were very slow. Stimulation of the hypogastric nerves now caused no contraction, the uterus remaining in a fully relaxed condition. A large dose of adrenaline still caused some rise in blood-pressure, and a contraction of the uterus occurred immediately after the injection, but this may have been spontaneous. It was followed by full relaxation lasting for nine minutes, and a new injection of adrenaline caused no contraction but a similar prolonged period of inhibition.

In this experiment hypogastric stimulation at first caused contraction of the uterus, but this effect was reversed by the injection of ergotoxine. The nerve stimulation now caused inhibition, and adrenaline and pilocarpine had the same effect.

These experiments indicate that the motor effects of pilocarpine on the pregnant uterus are antagonised by ergotoxine injected previously, while the inhibitory action is unimpaired by ergotoxine. In this again pilocarpine resembles adrenaline exactly, and in fact differs from it only in the former being antagonised by atropine, which has no effect on the latter. And not only is the motor action of pilocarpine abolished by atropine, but its effects on the inhibitory functions also disappear under it. When pilocarpine causes contraction of the uterus, atropine at once releases it. When it induces inhibition either directly or after ergotoxine, atropine reinstates the contractions only after some time, sometimes as long as 2—3 minutes elapsing before any movement occurs. This slow recovery from inhibition is not peculiar to pilocarpine, however, for even short stimulation of the hypogastric nerves with a weak current often induces inhibition lasting for many minutes afterwards.

The fact that the action of the pilocarpine on the uterus varies exactly with that of the hypogastric nerves suggests that the alkaloid affects some point on the path of the nervous impulse. And strong confirmation is afforded by the antagonism of large doses of ergotoxine

to the motor effects of pilocarpine and of nerve stimulation. On the other hand atropine, which antagonises pilocarpine completely, does not influence the passage of the nerve impulse or the action of adrenaline in any way; on the prevailing view that atropine antagonises pilocarpine by paralysing the point at which the latter acts, the nerve impulse would also fail to pass through this functionally inactive part of its course. Straub[1] has recently advanced the conception that pilocarpine exercises its influence on the contractile substance only in the course of its permeation into the cell and that atropine antagonises pilocarpine by retarding its permeation and thus reducing the potential of the inward current to subliminal dimensions. This would appear consistent with the fact that atropine antagonises pilocarpine in the uterus without interfering with the passage of the nerve impulse, but on the other hand fails to explain its simultaneous antagonism to pilocarpine and interference with the passage of the impulse in other organs such as the heart. And other physiochemical conceptions of the action of these alkaloids seem to promise no more satisfactory explanation of this curious complex at the present time. Returning to the view that pilocarpine affects some specific receptive substance and that atropine prevents its effects by acting on this substance either chemically or physically, the close analogy offered in my experiments between pilocarpine, adrenaline and nerve stimulation suggests that the receptive substances for pilocarpine are closely associated with the path of nerve impulse. And the antagonism of ergotoxine to all these agents as far as the contractor action is concerned indicates that these receptive substances must either be identical with those for ergotoxine or must discharge through the latter to affect the contractile substance proper. On the other hand the antagonism of atropine to pilocarpine alone seems to imply that the receptors for pilocarpine are not actually on the path of nerve impulse. The simplest mechanism which can satisfy the conditions observed appears to be a set of receptors for adrenaline and ergotoxine lying on the nerve impulse path and a separate and independent receptor for atropine and pilocarpine associated with the impulse path but not on the direct course to the contractile substance. This receptor can act on the contractile substance only as long as the adrenaline-ergotoxine receptive substance is intact and may be said to discharge through it. The relationship may be visualised by the nerve impulse path being represented as being joined above the adrenaline-ergotoxine receptor by a side branch on which

[1] *Pflüger's Arch.* CXII. p. 127. 1907.

the pilocarpine-atropine receptor lies. On the other hand, the receptor for atropine and pilocarpine in many other organs such as the heart, iris and salivary glands appears to lie on the nerve impulse path for the antagonism of pilocarpine by atropine involves the interruption of this path. It may be added that pilocarpine in some instances appeared to act less strongly on the inhibitory function of the uterus than adrenaline; for example it was observed in two experiments that while adrenaline brought the uterus to complete standstill for several minutes, pilocarpine merely caused marked relaxation but did not arrest the movements altogether.

The effect of pilocarpine in slowing the heart and reducing the blood-pressure was of course unaffected by the previous injection of ergotoxine in sufficient quantities to prevent any contraction of the pregnant uterus. In several experiments in which the uterine movements were being recorded, special attention was paid to the changes in the movements of the intestine under pilocarpine; these were not recorded graphically but merely watched in the bath. The marked peristalsis under pilocarpine is of course well known; after ergotoxine this pilocarpine peristalsis occurred in the same way as in the normal animal. Thus in experiments in which pilocarpine caused contractions of the pregnant uterus, intestinal peristalsis and slowing of the heart, the first only was eliminated by the subsequent injection of ergotoxine while the intestinal and cardiac changes persisted. A fresh injection of pilocarpine caused further slowing of the heart and more active peristalsis of the bowel, but the uterus was now inhibited. Atropine then accelerated the heart, induced contraction of the uterus and arrested the peristalsis of the bowel. Nothing could illustrate better the difficulties standing in the way of referring the effects of atropine (and of pilocarpine) to action on the ends of a definite group of nerves, as Loewi and Fröhlich have recently attempted. From a review of the effects of atropine, these observers limit the action of this alkaloid to paralysing the augmentor nerves of the cranial and sacral autonomic system, and in their final statement admit only one exception, in the sweat nerves. In the particular experiment to which I have last referred, no one of the three features of atropine action comes under this rubric.

The action of atropine and pilocarpine does not seem to me to be susceptible to definition by any such general rule. It is true that many of the most striking effects of atropine are due to its depressing the "terminations" of some of the augmentor cranial and sacral

autonomic nerves; but its sphere of action is not defined by these for it affects inhibitory cranial nerves (cardiac vagus) while it fails to paralyse cranial augmentor nerves (intestinal vagus), and it affects motor and inhibitory sympathetic functions (uterus, sweat-glands). It is true that in the case of some of the last it does not actually interrupt the path of nerve impulses, but it is impossible to dissociate entirely the action of atropine on the uterine mechanism from that on the heart and other organs.

The effect of atropine in preventing certain forms of motor excitation in the intestine, uterus and bladder, while not interfering with the normal movements and innervation of these organs, affords an explanation of its use in therapeutics to allay too powerful or too frequent contractions of these organs; the cause of these may perhaps be some abnormal product acting in the same way as pilocarpine and muscarine on the receptors which are affected by atropine.

In a number of experiments physostigmine was injected[1]. Kurdinowski[2] found that physostigmine caused powerful contractions of the uterus in the rabbit. In pregnant and non-pregnant cats I found active contraction induced by it. On the other hand I have not observed physostigmine cause any relaxation of the uterus either in pregnant or non-pregnant animals. And in some instances in which pilocarpine caused definite inhibition, physostigmine injection was followed by contractions. Thus in Fig. 4, *Es* indicates the injection of physostigmine salicylicate which is followed by powerful contraction of the uterus. Later pilocarpine (P) causes relaxation of the uterus, which is released by atropine (A) after which the organ assumed the same position as before the injection of physostigmine.

The effects of physostigmine on the uterus are antagonised by atropine, and I have not succeeded in restoring the contraction by subsequent injections of physostigmine. This seems to indicate that it acts not on the ultimate contractile substance but on a receptor similar to that affected by pilocarpine and perhaps identical with it. In one experiment, physostigmine failed to cause any movement after a large dose of ergotoxine, while pilocarpine was followed by relaxation and inhibition. And in no instance could I determine that physostigmine is capable of stimulating the inhibitory mechanism in the way described under pilocarpine. This indicates that while the receptors for pilocarpine are associated with both motor and inhibitory impulse paths to the uterus,

[1] Eserine salicylate (Merck) was employed.

[2] *Archiv f. Gynaekologie*, LXXVIII. p. 575. 1906.

those for physostigmine are associated with the motor path only or at any rate predominatingly.

The peripheral effects of physostigmine in other organs, except the striated muscles, seem generally to resemble those of pilocarpine closely, the differences being rather in degree than in kind. In the heart however physostigmine rarely causes such inhibition as is commonly seen from pilocarpine, though it apparently augments the effects of electrical stimulation of the vagus[1]. Here again the inhibitory impulse path seems to be less susceptible to the action of physostigmine than

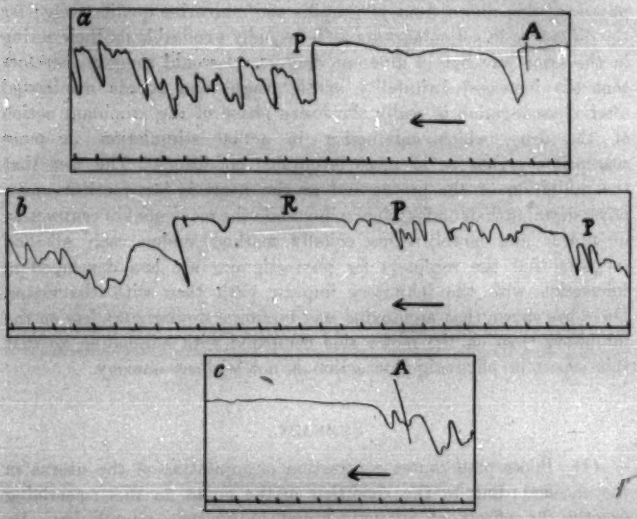


Fig. 5.

to that of pilocarpine. Loewi and Mansfield[2] have recently suggested that the peripheral action of physostigmine consists rather in augmenting the irritability of the terminations of the cranial and sacral autonomic nerves for normally subliminal stimuli than in acting as a stimulus itself; the grounds on which this view is based are that the effects of

[1] Winterberg. *Ztschr. f. exp. Path. u. Pharm.* IV. p. 36. 1907.

[2] *Arch. f. exp. Path. u. Pharm.* LXII. p. 180. 1910.

electrical nerve stimulation are augmented by the previous injection of physostigmine, while not infrequently no actual augmentor action is seen from physostigmine injection without subsequent electrical stimulation. The numerous exceptions to this observation they explain by a peripheral tone existing in certain organs. But this qualification of the theory seems to nullify the whole. It is undoubtedly true that not infrequently the injection of physostigmine is not followed by marked inhibition of the heart or by augmented salivary secretion, although the vagus and chorda nerves are found to respond by increased excitability; but this does not seem sufficient basis for the view that physostigmine differs from pilocarpine and muscarine qualitatively; for the difference in action appears to be equally explicable on their acting in the same way but in different degrees. I would suggest therefore that the increased irritability of the vagus and chorda manifested after physostigmine is really the lower phase of the stimulant action of the drug, which culminates in actual stimulation in more susceptible organs or in more favourable conditions. The fact that the inhibition of the uterus and of the heart is less marked under physostigmine than under pilocarpine, while the movement of contraction in uterus and bowel seems equally marked under each alkaloid suggests that the receptors for physostigmine are less developed in connection with the inhibitory impulse path than with the motor. Dale has shown that ergotoxine and tyramine similarly act less on the inhibitory than on the motor side compared with adrenaline, so that this aspect of physostigmine action is not without analogy.

Summary.

(1) Pilocarpine causes contraction or inhibition of the uterus in the cat according to the condition of the organ, in this resembling exactly the effects of adrenaline and hypogastric stimulation. Its contractor action is also antagonised by ergotoxine, as is that of adrenaline and electric stimulation.

(2) Pilocarpine differs from adrenaline in being antagonised completely by atropine whether it contracts or inhibits the uterus, while the effects of adrenaline or of hypogastric stimulation are not changed in any way by atropine.

(3) Physostigmine causes contraction of the uterus, and has no apparent effect in causing inhibition. It is antagonised by atropine completely.

(4) It is suggested that pilocarpine and atropine act on receptive substances which are associated with the nerve impulse path to the uterus, but do not actually lie on this path. Physostigmine may probably affect the receptors associated with the augmentor path only.

(5) In some other abdominal and pelvic organs, *e.g.* intestine, the receptor substances for atropine and pilocarpine appear to bear the same relation to the augmentor impulse path as in the uterus, while in other organs they must be regarded as lying on the impulse path, since this is interrupted by atropine.

THE COMPOSITION OF THE GASES OF THE BLOOD IN CHLOROFORM-ANÆSTHESIA.

By G. A. BUCKMASTER and J. A. GARDNER.

(*From the Physiological Laboratory, University of London.*)

Little attention has been paid to the changes in composition of the blood-gases in chloroform-anæsthesia, in spite of the well-known fact that, as the various stages of anæsthesia by chloroform which are clinically recognised succeed one another, the aspect of arterial blood to the naked eye becomes progressively venous in character. This change in colour must be due either to an altered ratio of oxy-hæmoglobin to reduced hæmoglobin, or to changes in form of the red corpuscles, which consequently reflect different amounts of light to the eye, as the observations of Harless in 1846 showed was possible when blood outside the body is saturated with oxygen or carbon-dioxide gas. The researches of Hamburger, G. N. Stewart and others have demonstrated that a many-sided exchange exists between the red corpuscles and plasma, which may be accompanied by an alteration in their volume and shape. When defibrinated blood is saturated with carbon-dioxide, some dissociated electrolytes pass from the corpuscles into the serum, and at the same time water enters the corpuscles, so that these individually become swollen, a phenomenon which is obvious by the alteration in the colour of the blood, which then becomes darker. Formanèk, Krüger and Edie have also shown that *in vitro* quantities of chloroform added to blood in amounts which produce no visible precipitation alter the colour.

Gangitano[1], who employed a spectro-photometric method, showed by periodic examinations, extending over many days, of the blood of

[1] F. Gangitano. *Arch. Ital. de Biol.* LI. p. 66. 1909.

patients who had been anæsthetised by chloroform, that the power of their hæmoglobin to fix oxygen was at first diminished, and this did not again become normal for several days. This change is not apparent to the naked eye. Moreover we have never been able to satisfy ourselves that the red corpuscles alter their shape either during anæsthesia or after death by chloroform. It is therefore probable that the darkened aspect of arterial blood is entirely connected with an altered gas-content. Satisfactory information on this point we have been unable to find.

Analyses have been published by Paul Bert[1], de St Martin[2], J. Tissot[3] and others, and also by Oliver and Garrett[4] of the gas-content of arterial blood during chloroform narcosis. The results appear to be somewhat conflicting.

Paul Bert considered that as anæsthesia proceeded the arterial blood became progressively poorer in oxygen and richer in carbon dioxide, and we quote one of his experiments on the gas-content of arterial blood in the dog under chloroform narcosis. Before anæsthesia the oxygen was 22·0 c.c. and the carbon dioxide 31·2 c.c. Thirty minutes later the oxygen had fallen to 16·8 c.c. and the carbon dioxide risen to 41·2. One hour later, and ten minutes before death, the value for oxygen was 14 c.c. and for carbon dioxide 44 c.c. De St Martin's analyses confirmed the opinions advanced by Paul Bert, though in some experiments he could find no diminution of oxygen at all in the arterial blood of chloroformed dogs.

The results obtained by J. Tissot only show that when the respiration stops in asphyxia induced by prolonged anæsthetisation arterial blood may contain only ·78 c.c. or 2·83 c.c. of oxygen, while the blood-gases in samples taken at intervals during anæsthesia by chloroform showed only a slight fall in oxygen-content so long as the ventilation of the lungs remained normal. Tissot concluded that during narcosis the blood retains to the time of death the same power of taking up oxygen, and if the amount of this diminishes in the blood, the decrease is solely due to a slowing of the respiration.

The experiments of T. Oliver and F. C. Garrett[4] were carried out on one dog and several rabbits, which were completely narcotised. For

[1] P. Bert. *Étude analytique de l'Anesthésie*, p. 144.

[2] St Martin. *Recherches expér. sur la respiration*, p. 189.

[3] J. Tissot. *Report of the Special Chloroform Committee of Brit. Med. Assoc.*, 1901–1902. Paper V, July 12, 1902.

[4] *Lancet*, Sept. 9, p. 695. 1893.

dog's arterial blood the figures were: CO_2 37·21 c.c.; Oxygen 17·09; Nitrogen 8·03 c.c.; $CHCl_3$ ·92 c.c. For rabbits' blood as follows:

	i.	ii.	iii.
CO_2	6·46	19·83	24·85
O	20·96	16·88	13·14
N	—	88·33	13·7
$CHCl_3$	54·72	4·55	2·59

Criticism of the foregoing experiments. We have shown[1] that the presence of chloroform introduces serious errors into the analyses of the gases of the blood which may often contain as much as 10 % of chloroform by volume, and consequently that the method of reducing the nitrogen values to 1·2 % and adjusting the oxygen accordingly is not justifiable[2]. The French observers make no mention of the presence of chloroform vapour and the analyses of Paul Bert give no figures for nitrogen at all. Thus, so far as can be judged, their results are necessarily unconvincing, and if so cannot be used as a basis for any views as to the gas-content of blood during chloroform anæsthesia. On the other hand, Oliver and Garrett were aware of the presence of chloroform vapour in the blood, and attempted to estimate its amount by combustion; but they made the mistake of combusting the residue *after* the absorption of the carbon dioxide by potash, and oxygen by ferrous tartrate. Some of the chloroform must have dissolved and been decomposed with the formation of carbon monoxide by the re-agents[3]. Their Pflüger pump obviously leaked considerably, and if the enormous nitrogen values are reduced to normal, and the oxygen values adjusted accordingly, the figures obtained are in general agreement with those which we have found in experiments that are described later in this paper.

Methods in which an attempt was made to eliminate the chloroform by absorption by neutral re-agents.

A variety of re-agents were tried, such as olive oil, vacuum oil, higher alcohols such as octyl alcohol, but all proved unsatisfactory, as they absorbed other constituents of the gas mixture besides chloroform. Paraffin wax of high melting point, cut into small spheres of about the size of a pea and with a perfectly smooth polished surface, proved less

[1] *Proc. Roy. Soc.* B, LXXXI. p. 516. 1909.
[2] This *Journal*, XL. p. 373. 1910.
[3] *Proc. Roy. Soc.* B, LXXXI. p. 525. 1909.

objectionable than the other re-agents tried. Preliminary experiments, in which measured volumes of chloroform-air mixture were passed into a Hempel pipette filled with paraffin balls and the resultant gases tested for chloroform by passing in a moist condition through a red-hot platinum spiral tube into silver nitrate solution, showed that, provided a sufficient surface of paraffin was used and sufficient time allowed for absorption, all the chloroform in the mixture could be got rid of. Unfortunately, however, the other constituents of the blood-gas were not unaffected by paraffin, but the oxygen was absorbed to a considerably less extent than the carbon dioxide. In the case of oxygen, the gas was run into a Hempel pipette filled with balls of paraffin; in one hour 0·13 % was absorbed, in 48 hours 2·4 %, in 52 hours 2·8 %, and in 76 hours 3·2 %. In a similar experiment with carbon dioxide, in 2 hours 4·1 % was absorbed, 10·3 % after 26 hours. We attempted to minimise this absorption by previously soaking the paraffin balls in a mixture of carbon dioxide and oxygen, but with only moderate success. It was evident therefore that if the chloroform vapour was eliminated in this manner and the blood-gases analysed in the ordinary way, the carbon dioxide would be too low and the chloroform be too high. The oxygen value would be approximately correct. A number of analyses were made by this method, the results of which are given below for what these are worth. The gases were extracted in the Toepler-Barcroft pump and the analyses made with Haldane's apparatus.

The results of the gas analyses are given in the following table; the nitrogen values are adjusted to about 1 % and the oxygen figures reduced accordingly, except in the case of Exp. I, which we give to show the figures actually obtained.

The volume of blood taken for a sample was 12·5 c.c. Strength of chloroform and air 2—3 %. This was administered through a tracheal cannula provided with Chauveau's valves, 10 to 15 c.c. of hirudin injected (except in Exp. IV) into femoral vein. We give Exp. III in detail. Continuous respiratory tracings were taken throughout the experiments.

III. Weight of cat 3·5 kilos.

45 mins.	Chloroform off.
47 ,,	Hirudin injected.
69 ,,	Reflexes all back.
	Chloroform on.
87 ,,	Reflexes all gone.
103 ,,	Sample A, 12·5 c.c.; blood very dark.
	Chloroform off.
	A sample taken at the same time was analysed by the method previously described by us[1], and found to contain 8·9 c.c. of chloroform vapour per 100 grammes of blood.
116 ,,	Eye reflexes back.
230 ,,	Sample B, 12·8 c.c.; blood bright red.

Volumes of gas per 100 c.c. *of blood.*

		Samples A						Samples B				
No. of Exp.	Mins. from starting chloroform	Total gas in c.c.	$CHCl_3$ vapour and CO_2 in c.c.		O. in c.c.	N. in c.c.	Reflexes well back, mins. after cessation of $CHCl_3$	Total gas in c.c.	$CHCl_3$ vapour and CO_2 in c.c.		O. in c.c.	N. in c.c.
I	34	57·99	46·24	CO_2 35·16 $CHCl_3$ 10·08	8·16	3·6	56	26·72	9·61	CO_2 8·83 $CHCl_3$ 0·78	13·26	3·75
II	51	37·28	31·89	CO_2 22·45 $CHCl_3$ 9·36	4·10	1·37	69	39·96	26·11	CO_2 24·35 $CHCl_3$ 1·76	12·85	1·00
III	34	47·93	40·47	CO_2 31·93 $CHCl_3$ 8·54	6·30	1·17	127	40·62	28·05	CO_2 25·52 $CHCl_3$ 2·53	10·99	1·56
IV	34	49·34	42·11	CO_2 33·67 $CHCl_3$ 8·44	6·24	1·00	89	36·68	21·57	CO_2 19·68 $CHCl_3$ 1·87	14·11	1·01

This method of experiment was finally discarded, because a number of observations showed that on making comparative analyses of chloroform-air mixtures by Waller's method and absorption by paraffin, the latter method uniformly yielded lower values, and the discrepancy was greater the higher the percentage of chloroform.

In another series of experiments the oxygen was first absorbed by means of phosphorus; the residual gas, consisting of chloroform vapour, carbon dioxide and nitrogen, was heated with strong potash until no further absorption took place; then mixed with oxygen and electrolytic gas in the well-known proportions, and exploded. The excess of oxygen was then absorbed by alkaline pyrogallate, and the residual nitrogen determined, the chloroform vapour and CO_2 being thus determined together by difference.

We give three experiments and the protocol of V when the animals were in the second stage of anæsthesia.

Tracings of the respiration were taken, both during and after the animal recovered from the anæsthetic. The results are given with the nitrogen values uncorrected.

V. Weight of cat, 3·3 kilos. Chloroformed in Bell-jar. Operation as in previous experiments.

35 mins. Hirudin injected into femoral vein.
53 ,, Sample taken, 12·5 c.c. blood very dark.

Volumes of gas per 100 *c.c. of blood.*

No. of Exp.	Hirudin	Total gases in c.c.	O in c.c.	CO_2 & $COCl_2$ in c.c.	N in c.c.	Corrected oxygen, c.c.
V	+	60·39	9·08	48·69	2·67	8·58
VI	0	61·20	7·48	50·13	3·6	6·79
VII	0	60·65	9·69	46·58	4·4	8·79

Method in which the blood-gas was analysed after combustion of the chloroform with excess of oxygen.

It did not seem feasible to explode with oxygen and electrolytic gas as, owing to the high percentage of chlorine in the chloroform, it was uncertain what the products would be; but it appeared likely that satisfactory results might be obtained by burning the chloroform with excess of oxygen in the presence of moisture by means of a red-hot platinum spiral in a manner similar to that used by Vernon Harcourt[1] for the estimation of chloroform. The apparatus used in the gas analyses was the well-known form of Frankland, as modified by W. A. Bone. For combusting the chloroform we used a pear-shaped glass bulb about 70 c.c. capacity, through the sides of which two stout platinum wires, connected by a spiral of fine platinum wire, were fused. The bulb was connected by a three-way tap with the measuring vessel of the gas analysis apparatus and with a small exit tube, through which water could be introduced. The bulb was also fitted with a separate mercury reservoir. The arrangement is shown in Fig. 1.

The gas to be analysed was measured, then mixed in the vessel *A* with a suitable quantity of oxygen, remeasured, and passed into the combustion bulb *B*, into which a few drops of water or copper sulphate solution had been previously introduced through the tube *C*. The chloroform was then combusted by heating the spiral to a bright red heat by means of an electric current for a period of from 40 to 60 minutes. After cooling and standing until all the hydrochloric acid produced had dissolved in the water, the diminution in volume was determined. The carbon dioxide was then absorbed by potash and the oxygen by alkaline pyrogallate in the usual manner.

We expected the burning of the chloroform to take place according to the equation given by Vernon Harcourt:

$$CHCl_3 + O + H_2O = CO_2 + 3HCl \quad \ldots\ldots\ldots\ldots\ldots\ldots (1).$$

[1] *Trans. Chem. Soc.* p. 1065. 1899.

Had this been the case, and the hydrogen chloride all dissolved in the water present, the volume of the chloroform present in the original gas mixture and the volume of the carbon dioxide produced by its combustion should have been respectively double the diminution in volume on combustion.

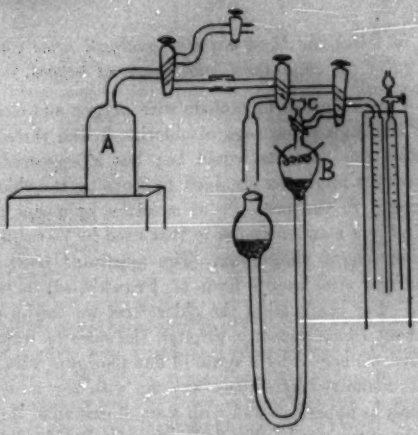


Fig. 1.

On carrying out the analysis as described above it was, however, noticed that a considerable quantity of calomel was deposited on the sides of the bulb, and the amount of the deposit did not appear to be affected by increasing the quantity of water. Mercury therefore appeared to enter into the reaction and the combustion appeared to be best represented by the equation

$$CHCl_3 + 2Hg + O_2 = CO_2 + 2HgCl + HCl \ldots\ldots\ldots\ldots (2).$$

According to this equation the volumes of the chloroform and the carbon dioxide produced by its combustion should respectively equal the diminution in volume.

In order to test the validity of this equation a large number of combustions of different mixtures of air or oxygen and chloroform were made, and the diminution in volume and the quantities of carbon dioxide produced were determined. The results, expressed in terms of

10 c.c. of gas measured at 0° C. and 760 mm., are given in the following table.

No. of Exp.	Volume of gas taken	Diminution in volume	CO_2 produced
(1)	10	0·77	0·67
(2)	,,	0·90	0·69
(3)	,,	0·55	0·65
(4)	,,	0·73	0·82
(5)	,,	0·60	0·79
(6)	,,	1·09	0·95
(7)	,,	1·07	0·87
(8)	,,	1·09	0·83
(9)	,,	0·81	0·82
(10)	,,	0·62	0·75
(11)	,,	0·64	0·78
(12)	,,	0·59	0·58
(13)	,,	0·47	0·61
(14)	,,	0·66	0·63
		10·59	10·44

The average value of the ratio of the diminution in volume to the carbon dioxide produced is therefore $\frac{1059}{1044} = 1{\cdot}014$. According to equation (1) this ratio should be 0·5, and according to equation (2) it should equal 1. Though the average ratio is very nearly 1, it will be noticed that the ratios in individual experiments show considerable variations in either direction. We have not determined finally the cause of this variation, but it is probably very largely due to the difficulty of getting a sharp separation of carbon dioxide and hydrogen chloride by means of the water.

The method of calculating the composition of a blood-gas from the analytical data will be best made clear by an example.

Pressure of gas at 0° C. and const. volume = 22·57 c.c., after addition of oxygen 32·21 cm., after combustion 30·53, after treatment with potash 15·98 cm., and after alkaline pyrogallate 0·57 cm. The oxygen added therefore measured 32·21 − 22·57 = 9·64 cm., the chloroform vapour 32·21 − 30·53 = 1·68 cm.; the carbon dioxide = 30·53 − 15·98 − 1·68 = 12·87 cm.; the oxygen = 15·98 − 0·57 − 9·64 + 1·68 = 7·45 cm.; the nitrogen = 0·57 cm.

As we have already pointed out, though the average ratio of the diminution in volume to the volume of CO_2 produced by combustion is practically 1, individual ratios often deviate from this very considerably in either direction. In any one experiment therefore the chloroform number given is liable to error, and can only be considered as approximately true, and this error also comes into the correction applied to the carbon dioxide and oxygen. As, however, the error in the correction

is small compared with the values corrected, it does not invalidate the result.

As it proved very difficult to keep the taps and joints of our Toepler pump in satisfactory order for a long series of experiments, we found it necessary to devise a special form of pump without taps or joints, the froth-chamber of which was continued downwards as a barometer tube, up which the blood was directly introduced. This pump has been fully described, together with its method of use[1].

Cats were used in all the experiments. Great care was taken to keep the temperature of the animals normal, since, in deep anæsthesia produced by chloroform and ether, the metabolism of the body is markedly depressed, and the thermotaxis of an animal is interfered with, so that any possibility of a warm-blooded animal passing into the condition of a cold-blooded one was as far as possible obviated. The temperature of an anæsthetised animal may fall as low as 22° C. We were careful to carry out the necessary operations with a minimum amount of interference and with absence of any hæmorrhage. A tracing of the respiratory movements was generally taken throughout the experiment. In some cases the percentage of chloroform administered was regulated by the Woulff bottle which we used in our previous work; in other cases by Waller's chloroform balance. The tracheal cannula was connected with Chauveau's valves. The blood from the vessel was always taken from the carotid artery, the cannula being introduced immediately before taking the sample. In some cases the blood ran from the animal through a capillary tube directly up the barometer tube into the froth-chamber. In other experiments the blood was collected by displacement of mercury in a bulb of known volume and again displaced up the barometer tube without coming in contact with the air. The details of these methods were fully described in our former paper[1].

In our experiments the main object was to compare the composition of the blood-gases of the normal animal with the composition on the one hand of the blood-gases at the stage when the reflexes vanished, and on the other hand the composition at a later stage of anæsthesia, when the animal was deeply anæsthetised, and in what we have previously described as the second stage of anæsthesia, when there is an equilibrium between the rate of absorption and the rate of elimination of chloroform by the blood[2]. It appeared to us that this informa-

[1] This *Journal*, XL. p. 373. 1910.

[2] *Proc. Roy. Soc.* B, LXXIX. p. 562. 1907.

tion would be obtained much more accurately by making a number of experiments on different animals at the different stages of anæsthesia, rather than by taking two samples at the different stages from each animal, because the withdrawal of the first amount of blood, sometimes a quarter of what the animal possesses, could not be without influence on the gas-content of the blood, since the absolute amount of hæmoglobin must be diminished, and the percentage of this would fall during a long experiment owing to the restoration of the blood-volume by an influx of lymph from the extra-vascular districts.

The results of our experiments as to the gas-content of the blood:

1. At the moment when the reflexes re-appeared,
2. When the reflexes disappeared,
3. During deep narcosis (second stage of anæsthesia),

are given in the following tables, and details of each experiment will be found at the end of this paper.

Experiments, in which the chloroform was administered for a very short time, just up to the moment of disappearance of the reflexes, and others in which these re-appeared during taking of the samples, are given in Table I. Respiratory tracings showed that the animals were breathing quietly and steadily.

TABLE I. *Composition of the blood-gases at 0° and 760 per 100 grammes of cat's blood at disappearance of reflexes.*

No. of Exp.	% of $CHCl_3$	Time in mins. from commencement of $CHCl_3$	Hirudin 10 c.c. injected	Amount of blood in c.c.	Colour	Total gas in c.c.	$CHCl_3$ in c.c. vapour	CO_2 in c.c.	O. in c.c.	N. in c.c.
A	2—3	3	+	29	Bright	43·65	3·32	27·76	9·52	3·05
B	—	5	+	29	Slightly	38·49	3·88	26·45	6·12	2·03
C	1—2	23	0	28·5	Fairly Bright	45·96	2·36	34·51	7·71	1·38

Remarks:—Exp. A. Reflexes re-appear during taking of sample. Oxygen used for combustion not quite free from nitrogen.

Exp. B. Eye-reflexes and patellar back during taking of sample. Oxygen used contained a trace of nitrogen.

Exp. C. Chloroformed very slowly. Eye and patellar tendon just gone, not tail reflex.

In Table II we give the results in which the animal received chloroform in a bell-jar for a considerable length of time, and when fully anæsthetised was removed for the necessary operation; the administration continued for a variable length of time, and the sample was taken when the reflexes re-appeared after cessation of the

anæsthetic. Although a considerable quantity of blood was abstracted, the colour showed that it was bright red arterial blood. The animals were respiring somewhat faster than when fully under the anæsthetic.

TABLE II. *Composition of the blood-gases at* 0° *and* 760 *per* 100 *grammes of blood at reappearance of reflexes.*

No. of Exp.	% of $CHCl_3$	Time in mins. from commencement of $CHCl_3$	Hirudin 10 c.c. injected	Amount of blood in c.c.	Colour	Total gas in c.c.	$CHCl_3$ in c.c. vapour	CO_2 in c.c.	O in c.c.	N in c.c.
D	—	20	+	48·6 gr. = 46·11 c.c.	—	46·67	3·47	26·62	15·41	1·18
E	1—2	45	+	76·65 gr. = 72·72 c.c.	Somewhat dark	40·54	0·89	26·31	12·14	1·19
F	2	Intermittent for a long time		10·8	Bright red	42·00	4·19	30·31	5·82	1·68
G	—	35	+	47	Bright red	49·96	3·27	32·83	12·58	1·28

Remarks:—Exp. D. Sample on re-appearance of reflexes.
Exp. E. Sample just before re-appearance of reflexes.
Exp. F. Chloroform repeatedly on and off; occasionally artificial respiration. Reflexes back when sample taken.
Exp. G. Eye, patellar and tail back.

In Table III the composition of the blood-gases in arterial blood, in that condition of narcosis which we have called the second stage of anæsthesia, is given.

TABLE III. *Composition of the blood-gases at* 0° *and* 760 *per* 100 *grammes of blood in the second stage of anæsthesia.*

No. of Exp.	% of $CHCl_3$	Time in mins. $CHCl_3$	Hirudin	Amount of sample, c.c.	Colour	Total gas	$CHCl_3$ in c.c. of vapour	CO_2	O	N
H	1·5–2	56	+	29	Dark	44·52	1·38	36·73	4·06	2·35
I	2	33	0	22	Dark	57·37	3·55	48·38	3·51	1·84
J	2	45	0	51·23	Fairly dark	33·67	2·89	16·98	11·63	1·17
K	2	60	+	95·83	Very dark	53·99	5·95	36·79	9·90	1·34
L	2	55	0	47	Very dark	56·69	5·27	41·66	8·58	1·16
M	2	100	+	47	Very dark	58·48	6·51	39·73	10·63	1·38
N	2	63	+	47	Very dark	46·21	6·92	29·91	8·12	1·26
O	2	76	+	47	Very dark	53·37	5·68	37·78	8·50	1·40

Remarks:—Exp. H. Respiration regular and steady. Animal very slowly anæsthetised with a low percentage of $CHCl_3$.
Exp. J. Animal not far beyond reflex point.
Exp. N. Not far from asphyxial point.

In Table IV the composition of normal cat's blood is compared with that of the same species of animal:

1. When the reflexes re-appear after anæsthesia.
2. When the reflexes just disappear.
3. In the second stage of anæsthesia.

TABLE IV.

	Average volume in c.c. per 100 c.c. of blood			Average composition % of gas			
	CO_2	O	N	CO_2	O	N	Ratio of O to CO_2
Normal cats[1]	25·07	13·60	1·00	63·2	34·28	2·52	1 : 1·84
Reflexes just re-appear	29·02	11·49	1·33	65·06	25·44	2·87	1 : 2·55
Reflexes just disappear	29·57	7·78	2·15	69·14	18·17	5·09	1 : 3·8
2nd stage of anæsthesia	36·00	8·14	1·49	71·27	16·12	2·95	1 : 4·42

Discussion of Results.

The total gases found in the blood of normal cats, as was pointed out by Pflüger, is low compared with that of dogs. A hundred grams of arterial blood, we found in six experiments, yielded an average amount of 39·68 c.c. of mixed gases[2]. In animals slightly anæsthetised with chloroform the average amount of gas in six experiments was 43·74 c.c., and in cats completely anæsthetised it was 50·5 c.c.; an increase per cent. of 10·2 and 26·2 respectively. It is evident from the tables that the increase is chiefly due to an augmented amount of carbon dioxide gas.

In a former paper[3], we showed that the chloroform-content of the blood rises in the initial stages of anæsthesia with great rapidity to a value which approaches the maximum. During this period the amount of chloroform in the blood appears to chiefly affect the respiratory centres, so that the breathing becomes slower and sometimes stops altogether. If the animal passes this stage naturally, or recovers on cessation of the administration of chloroform, or by means of artificial respiration, then, on continuing the anæsthetic the amount of chloroform in the blood quickly rises again towards a maximal value and an equilibrium between the factors which determine the amount of chloroform in the blood appears to be established, the processes of intake and output at the pulmonary surface going on side by side.

[1] Buckmaster and Gardner, This *Journal*, XLI. p. 60. 1910.
[2] *Ibid.* [3] *Proc. Roy. Soc.* B, LXXIX. p. 535. 1907.

The following curve is constructed from data given in the paper we have quoted.

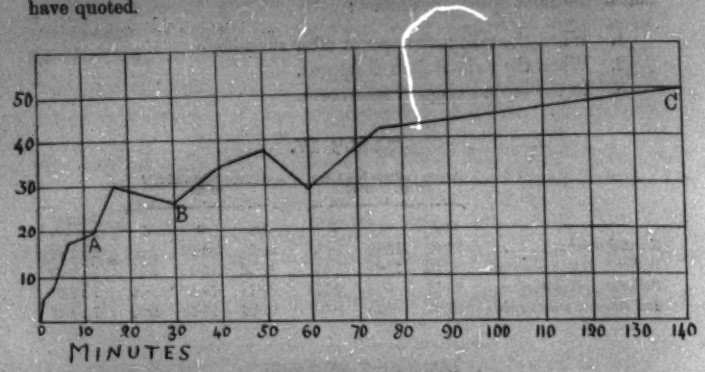


Fig. 2. Curve showing the chloroform-content of arterial blood of the cat during anæsthesia.

A, points at which the eye-reflexes vanish.
B to C, complete anæsthesia, second stage.

The vertical row of figures expresses the content of the blood in milligrams of chloroform in 100 grams of blood.

In Tables I and II the average amount of chloroform in milligrams per 100 grams of blood is 16—17. In Table III the amount is 26. The stages of anæsthesia at which the samples of blood were taken for gas-analysis will therefore be clear from the above curve.

It is obvious that the darkened colour of blood in chloroform narcosis is due to a diminution in the amount of oxy-hæmoglobin. In normal cats the amount of hæmoglobin by Gowers-Haldane hæmoglobinometer lies between 70 and 80, which, in terms of the percentage oxygen capacity, would give a value slightly higher than 13·6, which is the average volume of oxygen found by gas-analysis. The fall in oxygen-content during narcosis is well marked, even when the reflexes disappear; while, in the second stage of anæsthesia, the reduction is 40 %. The hæmoglobin is therefore only partially saturated with oxygen during narcosis, indeed to the extent of 60 %. The blood of the normal cat, on the assumption that the alveolar air contains 14 % of oxygen, should, when allowance is made for the tension of aqueous vapour at 38° C., = 49·3 mm., with 40 mm. CO_2 tension in blood, be saturated to the extent of 90 %, the oxygen tension being equal to

99·49 mm. But during narcosis the hæmoglobin is in the same state of partial saturation that it would be with a carbon dioxide tension of 40 mm. and an oxygen tension of only 45·5 mm. For it is probable since the salts in the red corpuscles in both the blood of dogs and cats are alike in containing chiefly salts of sodium, that the curve of dissociation of dogs' blood given by Barcroft and Camis[1] is applicable to the blood of cats.

Tracings of the respiratory movements taken during each of the experiments give no indication that this is to be attributed to slowing of the respiration. It is not necessary to reproduce these tracings in this paper, they all conform to those we have already published at full length[2]. An examination of these curves shows that in no case, during the second stage of anæsthesia, is there any marked alteration in the frequency or character of the respiration; certainly *there is not any slowing of the rate or depth* of the respiratory movements. We may therefore infer that the lung-ventilation is not interfered with, and are consequently quite unable to agree with the view expressed by J. Tissot,[3] from the results of three experiments and adopted in the Report of the Chloroform Committee of the British Medical Association[4] 1910, "that during narcosis, the blood retains up to the time of death the same power of taking up oxygen, and that if the amount of this gas diminishes in the blood, the decrease is solely due to a slowing of the respiration."

It is known that chloroform is associated with the red corpuscles, and outside the body aggregations of hæmoglobin and chloroform have been described[5], which are said to possess distinctive spectra[6]. It is therefore probable that the fall in oxygen-content of the blood in chloroform narcosis, is due to direct interference with the single function of transporting oxygen from the lungs, which the red corpuscles possess.

[1] J. Barcroft and M. Camis. This *Journal*, XXXIX. p. 132. 1909.

[2] G. A. Buckmaster and J. A. Gardner. *Proc. Roy. Soc.* B, XXIX. p. 577. 1907.

[3] *Report of Special Chloroform Committee of the Brit. Med. Assoc.* Paper V. 1901–2.

[4] *Final Report of Special Chloroform Committee of the Brit. Med. Assoc.* p. 52, July 9. 1910.

[5] B. Moore and Roaf. *Proc. Roy. Soc.* LXXIII. 1904, and LXXVII. 1905. Eadie, *Reports of Thompson Yates and Johnston Laboratories, Liverpool*, VI. 1905.

[6] F. Krüger. *Hofmeister's Beiträge*, III. p. 67. 1903.

Protocols.

A. Cat, weight 2·2 kilos. Animal killed by a blow on the head.

5th min. 10 c.c. hirudin in femoral vein.
12th ,, $CHCl_3$ 2 % on (Woulff's bottle).
15th ,, Eye-reflexes gone, also patellar tendon. $CHCl_3$ off.
Sample taken 29 c.c. medium dark colour.
Reflexes returned during taking of sample.

Pressure of gas at constant volume (72·48 c.c.) = 18·8 cm. at 10·8° C.: after addition of O. = 40·35 cm. at 10·6° C.: after combustion = 39·55 cm. at 12·6° C.: after potash 29·65 cm. at 12·5° C.: after alkaline pyrogallate 1·90 cm. at 11·8° C. The oxygen used contained 3·5 % of nitrogen.

B. Cat, weight 3·2 kilos. As Exp. A.

7th min. Hirudin 11 c.c. into femoral vein.
11 ,, $CHCl_3$ on.
14 ,, Eye-reflexes gone, then patellar tendon.
14·5 ,, $CHCl_3$ off. Sample taken. Eye-reflexes back during taking of sample. Blood slightly dark.

Pressure of gas at constant volume (72·48 c.c.) = 12·35 cm. at 15·1° C.: after addition of O. = 35·20 cm. at 15·4° C.: after combustion = 33·80 cm. at 14·1° C.: after potash 14·10 cm. at 14·1° C.: after pyrogallate 0·65 cm. at 14·1° C.

C. Exp. as A and B. No hirudin.

6th min. Reflexes present, $CHCl_3$ on.
6·5 ,, $CHCl_3$ off.
7 ,, $CHCl_3$ on very weak (1 %).
28 ,, Sample taken. Eye and patellar reflexes gone, but not tail. Vol. of blood 28·5 c.c.

Pressure of gas at constant volume (72·48 c.c.) = 14·4 cm. at 13·2° C.: after addition of O. 25·15 cm. at 13·1° C.: after combustion 24·30 cm. at 11·8° C.: after potash 12·80 cm. at 11·7° C.: after pyrogallate 0·43 cm. at 12° C.

D. Cat, weight 2·5 kilos. $CHCl_3$ in bell-jar.

15th min. Hirudin 10 c.c. in femoral vein.
20 ,, Sample. Reflexes just gone. Weight of blood 48·6 grammes = 46·11 c.c. Hb = 93 % (Gowers-Haldane).

Pressure of gas at constant volume (72·48 c.c.) = 23·6 cm. at 12·5° C.: after addition of O. = 39·7 cm. at 12·6° C.: after combustion 31·9 cm. at 12·3° C.: after potash 16·75 cm. at 13·2° C.: after pyrogallate 0·60 cm. at 12·7° C.

E. Cat, weight 3·2 kilos. $CHCl_3$ in bell-jar.

25th min. Hirudin 15 c.c. injected into femoral vein.
55 ,, Sample taken 76·65 grammes = 72·72 c.c. just before reflexes re-appeared, but these appeared during withdrawal of blood, which was somewhat dark. Hb = 77 % (Gowers-Haldane).

Pressure of gas at constant volume (72·48 c.c.) = 32·15 cm. at 10·9° C.: after addition of oxygen = 42·35 cm. at 11·3° C.: after combustion = 41·85 cm. at 12·8° C.: after potash 20·05 cm. at 11·5° C.: after pyrogallate 0·95 cm. at 12·3° C.

F. Cat, weight 4 kilos. $CHCl_3$ in bell-jar.

$CHCl_3$ 2 % on and off intermittently, and artificial respiration at times. No hirudin.

Sample bright red, when reflexes back, 10·6 c.c. Hb = 71 % (Gowers-Haldane).

Pressure of gas at constant volume (72·48 c.c.) = 5·0 cm. at 14° C.: after oxygen = 30·7 cm. at 14·1° C.: after combustion 30·2 cm. at 14·1° C.: after potash 26·1 cm. at 14·2° C.: after pyrogallate 0·7 cm. at 14·8° C.

G. Cat, weight 2·9 kilos. $CHCl_3$ in bell-jar.

22nd min. Tracheal cannula in, $CHCl_3$ on.
35th ,, Hirudin 10 c.c. into femoral vein.
45 ,, Sample for pump 47 c.c. Blood bright red. Eye, patellar tendon and tail reflexes present.

Pressure of gas at constant volume (72·48 c.c.) = 25·68 cm. at 11·7° C.: after addition of oxygen = 44·24 cm. at 11·4° C.: after combustion = 42·52 cm. at 11·1° C.: after potash = 24·01 cm. at 11·1° C.: after pyrogallate = 0·66 cm. at 11·1° C.

H. Weight of Cat 2·7 kilos. $CHCl_3$ in bell-jar.

25th min. Table. Eye reflexes present. $CHCl_3$ on, weak 1·5 %—2 %.
38 ,, Eye reflexes still present.
42nd ,, Eye reflexes gone. Respiration regular and steady.
50th ,, Hirudin injected.
56 ,, Sample taken. Blood dark, 29 c.c. Hb = 80 % (Gowers-Haldane).

Pressure of gas at constant volume (72·48 c.c.) = 14·3 cm. at 15·4° C.: after oxygen 24·95 cm. at 15·1° C.: after combustion 24·25 cm. at 12·1° C.: after potash 12·2 cm. at 13·2° C.: after pyrogallate 0·75 cm. at 13·6.

I. Cat, weight 3·9 kilos. $CHCl_3$ in bell-jar.

12th min. Tracheal cannula in, $CHCl_3$ on 2 %.
30 ,, Cannula in carotid artery.
33rd ,, Sample. 22 c.c. Dark coloured blood.

Pressure of gas at constant volume (72·48 c.c.) = 14·0 cm. at 15·8° C.: after oxygen = 25·4 cm. at 15·8° C.: after combustion 24·6 cm. at 16·6° C.: after potash 11·9 cm. at 16·8° C.: after pyrogallate 0·45 cm. at 16·7° C.

J. Cat, weight 3·5 kilos. $CHCl_3$ in bell-jar.

15th min. Hirudin injected.
45 ,, Blood sample fairly dark. 54 grammes = 51·23 c.c. Hb = 75 % (Gowers-Haldane).

Pressure of gas at constant volume (72·48 c.c.) = 18·3 cm. at 11·7° C.: after oxygen = 27·65 cm. at 10·1° C.: after combustion 25·95 cm. at 9·1° C.: after potash = 14·90 cm. at 8·7° C.: after pyrogallate = 0·65 cm. at 8·5° C.

K. Cat. Hirudin. $CHCl_3$ in bell-jar.

15th min. Tracheal cannula in, $CHCl_3$ on from Woulff's bottle.
60 ,, Sample very dark. 101 grams = 95·83 c.c. Hb = 68 % (Gowers-Haldane).

Pressure of gas at constant volume (72·48 c.c.) = 56·2 cm. at 9·8° C.: after oxygen = 70·9 cm. at 9·5° C.: after combustion = 65·2 cm. at 11·6° C.: after potash 20·4 cm. at 11·3° C.: after pyrogallate 1·4 cm. at 9·1° C.

L. Cat, weight 2·8 kilos. $CHCl_3$ in bell-jar. No hirudin.

20th min. Tracheal cannula in, $CHCl_3$ on.
55 ,, Sample. Blood very dark. 47 c.c.

Pressure of gas at constant volume (72·48 c.c.) = 28·99 cm. at 10·3° C.: after oxygen = 43·79 cm. at 10·6° C.: after combustion = 41·09 cm. at 10·3° C.: after potash = 17·09 cm. at 10·3° C.: after pyrogallate = 0·60 cm. at 10·3° C.

M. Cat, weight 3·3 kilos. $CHCl_3$ in bell-jar, hirudin.

20th min. Tracheal cannula in, $CHCl_3$ on.
100 ,, Sample taken 47 c.c. Blood very dark.
Samples taken for estimation of $CHCl_3$ by Nicloux' method = 59 mmgrs. of $CHCl_3$ per 100 grams of blood.

Pressure of gas at constant volume (72·48 c.c.) = 30·03 cm. at 11·5° C.: after addition of oxygen = 39·15 cm. at 10·6° C.: after combustion = 35·72 cm. at 9·8° C.: after potash = 12·09 cm. at 9·8° C.: after pyrogallate = 0·71 cm. at 11·3° C.

N. Cat. $CHCl_3$ in bell-jar, hirudin.

19th min. Tracheal cannula in, $CHCl_3$ on.
63 ,, Sample taken. Very dark not far from asphyxial point, 47 c.c.
Sample before and after gave by Nicloux' method ·45 and ·33 mmgrs. of $CHCl_3$ per 100 grams of blood.

Pressure of gas at constant volume (72·48 c.c.) = 23·81 cm. at 12·4° C.: after addition of oxygen = 43·48 cm. at 12·1° C.: after combustion = 39·96 cm. at 12·4° C.: after potash = 21·08 cm. at 13·8° C.: after pyrogallate = 0·65 cm. at 13·8° C.

O. Cat. $CHCl_3$ in bell-jar. Hirudin.

10th min. Tracheal cannula in, $CHCl_3$ on.
76 ,, Sample, very dark in colour, 47 c.c.

Pressure of gas at constant volume (72·48 c.c.) = 27·31 cm. at 10·5° C.: after addition of oxygen = 46·00 cm. at 9·7° C.: after combustion = 43·30 cm. at 11·0° C.: after potash = 20·97 cm. at 10·4° C.: after pyrogallate = 0·72 cm. at 11·6° C.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for help in carrying out this work.

VISUAL PHENOMENA CONNECTED WITH THE YELLOW SPOT.

By F. W. EDRIDGE-GREEN, M.D., F.R.C.S., *Beit Medical Research Fellow*[1].

(*From the Institute of Physiology, University College, London.*)

I PROPOSE considering a certain number of subjective and entoptic phenomena connected with the yellow spot.

I. *Various appearances in the field of vision due to peculiarities of the yellow spot.*

1. *Loewi's experiment.* If we look at a clear white surface through a solution of chloride of chromium, which is of a celadon green colour, we shall see a purple spot in the centre which varies in size and shape with different persons. On careful examination it will be found to consist of three portions, the centre corresponding to the fovea appears as a bright purple disk, the middle corresponding to the non-vascular portion of the yellow spot appearing as a dark green ring, and lastly Loewi's ring corresponding more or less accurately to the outer feeble yellow portions of the yellow spot which appears as a purple ring surrounding the central portion and possessing a diameter twice or three times as large.

2. *Apparent size of regions of yellow spot in field of vision.* The region corresponding to the yellow spot occupies a considerable area in the field of vision. I find that the visual angle which any portion subtends can be easily measured by projecting the after-image of an object occupying a known visual angle on the sky above a house. A comparison is easily made in both cases with portions of the house. If an observer look straight at the cloudless sky, preferably about three hours before the sun sets, for about ten to twenty seconds, a disk or an ellipse with the long diameter horizontal and which is to me of about nine degrees appears in the sky. Inside this is a central darker portion of about two degrees in each diameter and this surrounds a central

[1] A paper read in the Phys. Section of the Brit. Med. Ass. 1910.

brighter portion which corresponds to the point of direct vision. This is about 40 to 50 minutes in diameter and is circular in form. See Fig. 1.

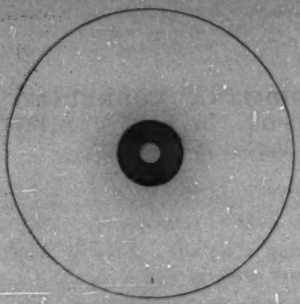


Fig. 1.

Helmholtz[1] gives a disk of the diameter of about 40 to 50 minutes as the apparent size of the fovea in the field of vision, and the diameter of the aureola which corresponds to the non-vascular portion of the yellow spot and is the portion where the yellow is most intense as about two degrees. This is correct for me. The remaining portion which corresponds more or less exactly to the remainder of the yellow spot is Loewi's ring.

3. *Appearance of central region with different intensities of light.* Exner[2] points out that with feeble light the central region is seen as a dark disk surrounded by Loewi's ring and that as the intensity is increased this region becomes brighter and brighter until it becomes more luminous than its surroundings, the fovea being specially noticeable as a bright disk of greater intensity than any other part.

The appearance of Loewi's ring varies with different persons and in different conditions. Loewi saw the ring circular and so do I but Helmholtz saw it rhomboidal. I see all three portions circular in the conditions I have just mentioned and every part is darker than the surrounding sky, the region corresponding to the fovea being the brightest of the three. I have seen after looking at the sky the central portion of the yellow spot appear as a bright yellow spot with a dark centre. It was circular in form and on one occasion lasted for ten minutes before disappearing.

[1] *Physiol. Optik*, p. 567.

[2] *Ctrlb. f. d. Med. Wissnsch.* 8, 594. 1868.

When the light is feeble the foveal region is not seen and the central dark portion is larger and Loewi's ring is smaller. The shape is then more often oval, rhomboidal, or irregular. Fig. 2 shows the appearance to my right eye on a dark night with a cloudless sky. The dark centre was surrounded by a lighter portion. Bright lines then came from the periphery and proceeded in an irregular manner to the centre. When these lines reached the centre each broke up in a very similar way to a rocket and left the surrounding portion lighter than before. This continued until the yellow spot region became lighter instead of darker than the ground.

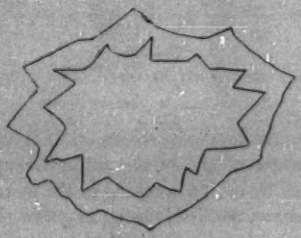


Fig. 2.

I have seen the yellow spot region largest and darkest when on waking in the early morning I have directed my eyes to the white ceiling. The whole of the central portion of the field of vision appeared as a black disk surrounded by luminous circles. These circles then interlaced and encroached on the central black portion which disappeared from without inwards.

There are several ways in which the region of the yellow spot may be seen bright on a dark ground. Helmholtz[1] has seen it on waking in the morning and looking at a dark background after having first exposed the eyes to the light from a large window. A disk of dazzling brightness is seen of the size of the non-vascular areola of the yellow spot.

I find that the region corresponding to the non-vascular portion of the yellow spot can be seen very well as a bright spot on a dark ground in the following way. If one eye be closed and the other directed to the sky through a deep red glass, after ten or twenty seconds the

[1] *Optik Physiol.* S. 869.

central portion will appear purple instead of red. If the eye be now closed and covered with the hand so as to exclude all light, the field of vision appears green with the exception of the centre which is seen as a bright red spot, much brighter than the rest of the field. The green gradually invades this red from without inwards until the whole field of vision is one uniform green and the centre becomes of similar brightness to the parts surrounding it. See Fig. 3.

Fig. 3.

Fig. 4 shows a sector of one of the subjective appearances of the central region as seen by me on awaking in the morning. The centre has a spotted appearance, the circles being larger at the periphery, with gradually increasing black intervals. Outside the macular region the spots of light are further apart and less defined.

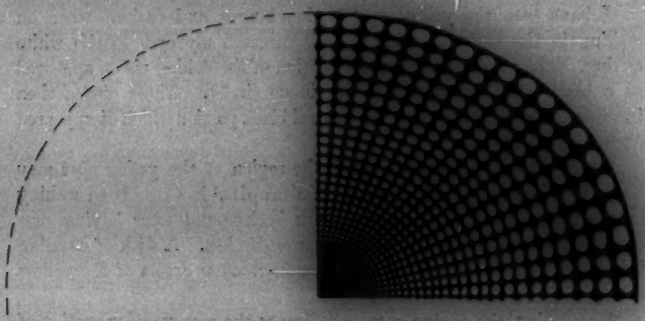


Fig. 4.

The retina after a night's rest is in a most favourable state for entoptic experiments. On awaking in the morning and then directing the eyes to a white ceiling the region corresponding to the yellow spot

is marked out as an oval black patch, and light appears to invade this patch from without inwards. Helmholtz concludes from this that light is perceived an instant later in the central as compared to the peripheral portions of the retina. Helmholtz attributes this observation to Maxwell[1] but as a matter of fact Maxwell said exactly the opposite. He says: "If we look steadily at an object behind a series of bright bars which move in front of it, we shall see a curious bending of the bars as they come up to the place of the yellow spot. The part which comes over the spot seems to start in advance of the rest of the bar, and this would seem to indicate a greater rapidity of sensation at the yellow spot than in the surrounding retina."

I agree with both these contradictory statements.

II. *Entoptic appearance of the yellow spot and the blood vessels of the retina.*

The yellow spot and the blood vessels of the retina may be seen entoptically in several ways. Purkinje described the first three methods[2].

1. *Illumination through sclerotic.* If by means of a lens of short focus a light, as intense as can be endured without discomfort, be concentrated on the sclerotic as far as possible from the cornea whilst the eye is directed towards a dark surface the vessels of the retina will be plainly visible on a yellowish red background. The finest capillaries can be seen and it will be noticed that these are absent in the centre of the field of vision, the vacant space being bordered by the loops of the capillaries. Whilst the portion of the field of vision corresponding to the rest of the retina appears of a uniform illumination, that in the centre where there are no capillaries is brighter and has an appearance similar to that of the fovea under the microscope in which the cones are seen as a series of small round circles, when the retina is viewed from its external side. See centre of Fig. 4. Helmholtz describes the entoptic appearance of this central non-vascular portion as being similar to that of shagreened leather. If the light be moved upwards on the sclerotic whilst the eye is kept fixed the images of the vessels appear to move in the same direction whilst the central non-vascular portion appears to move slightly in the opposite direction. Helmholtz after showing in a very complete manner how the shadows of the arteries

[1] *Report of Brit. Assoc.* II. p. 12. 1856.

[2] *Btr. z. Kenntn. d. Sehens*, S. 49. 1819. *Neue Btr.* S. 115, 117. 1825.

cause the effects which are seen, remarks that the appearance of the central portion is undoubtedly not produced in the same manner. It will be noticed that the vascular tree encroaches on the central portion on the side opposite the light whilst above and below it only touches it; on the side nearest the light there is an interval between the two. All the appearances remain the same whether the light be at the internal or external angle of the eye. The reason of this is that the vascular system is situated anteriorly to the portion of retina which gives rise to the spotted appearance. The portion of retina which gives rise to this appearance exactly corresponds to the non-vascular portion of the retina. When I have repeated this experiment, and I have found the light from an acetylene lamp concentrated on the sclerotic answer admirably, the central portion has first appeared dark but in addition to the vessels concentric bluish-violet coloured waves are seen. These waves appear as bluish-violet coloured circles of light which roll inwards from the outer part of the field of vision. They occupy the whole circumference and appear as steadily diminishing circles. Each succeeding circle reaches further towards the central portion of the field of vision until one touches it. It then appears to break up into a star-shaped figure and becomes much brighter. This is then replaced by the spotted appearance already mentioned.

J. H. Nuel[1] and L. Wolffberg[2] have come to the conclusion from careful measurements that the appearance of the central portion corresponds to the mosaic formed by the cones of the fovea.

2. *Illumination through cornea.* The second method of seeing the blood vessels of the retina is similar to the first only the light is allowed to enter the eye through the cornea. The observer being in a darkened room and the eyes being directed forward a candle is moved backwards and forwards either at the side or above the eye. The fine vessels and capillaries are not seen so well in this method as in the other two. In the central portion several observers have seen a bright disk circular or elliptical in shape, others have been unable to see the whole of the disk. In ordinary circumstances I only see a portion of the disk, it is bordered on its outer side, the side nearest the light, by a dark crescent and external to this is a bright crescent which is much brighter than any portion of the interior of the disk. When the light is moved upwards the bright crescent also appears to move upwards. When I move the light much more quickly and with a circular movement I am able to see

[1] *Arch. de Biol.* T. IV. 1883.
[2] *Arch. für Augenhl.* XVI. 1886.

the whole of the disk. The appearance then often changes to that seen in the first method, this appearance being generally preceded by the pale bluish-violet circles. I often find that the central portion appears dark and not light. I find that the central portion appears dark with red light whilst with green light it appears bright and spotted, the rest of the field also appears brighter.

3. *Illumination through pinhole.* The third method of seeing the retinal vessels consists in viewing a large uniformly illuminated surface, as for instance the sky, through a pin hole aperture which is moved rapidly backwards and forwards in front of the eye. The capillaries are very clearly defined, dark on a light ground. In the centre there is a portion free from vessels and bounded by the convex side of the loops of the capillaries.

4. *Illumination when the retina is specially sensitive.* I find that I can see the retinal vessels very clearly if on opening my eyes in the morning on awaking I immediately direct them to the white ceiling. The larger vessels appear very black and distinct. The central portion of the field of vision appears as an oval black patch and light appears to invade this patch from without inwards. The effect is only momentary.

III. *Currents seen in the field of vision not due to the circulation.*

1. *Currents seen with one eye partially covered.* If one eye be partially covered with an opaque disk whilst both eyes are directed forwards in a not too brightly illuminated room and special attention be paid to the covered eye an appearance of whirling currents will be seen with this eye. See Fig. 5. These currents appear to be directed towards the centre and have a very similar appearance to a whirlpool. On closing both eyes all the portion in which the whirling currents are seen appears as dull purple. These currents cannot be due to vessels because we know that the centre of the retina, corresponding to the point where the greatest movement is seen, is free from vessels. The appearance is also very different from that of the movement of blood in vessels. The experiment succeeds best if the eyes have been previously exposed to a fairly bright light. An opaque disk in a spectacle frame suffices admirably, a certain amount of light being allowed to enter the eye from the periphery.

2. *Currents seen in the light with one or both eyes open.* It is easy to see the currents at almost any time on regarding fixedly a not too

brightly illuminated surface. I find that it is better to use only one eye but they can be seen with both eyes open. The first appearance which is always visible to me is a star-shaped figure corresponding to the region of the fovea. Fig. 6. When seen with one eye this star has eight rays. It is due to the structure of the lens. There appears to be rapid

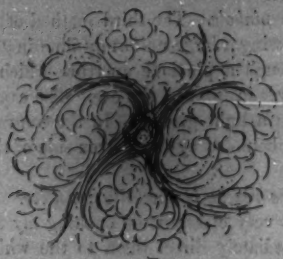


Fig. 5.

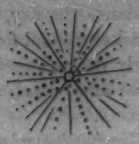


Fig. 6.

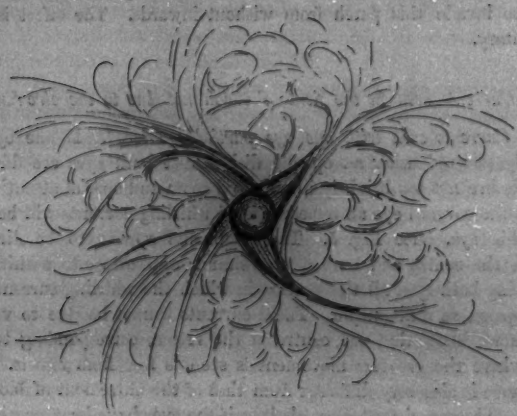


Fig. 7.

circular movement behind this star, that is, the movement appears to be further off in the field of vision, like a top spinning or a catherine wheel, the rays of the star remaining always visible and stationary. The movement is from left to right with the right eye and from right

to left with the left eye. The field of vision then becomes dark and the movement spreads until it covers the whole region corresponding to the yellow spot and the currents in this outer portion form a network with wide meshes. Fig. 7. The currents seem to proceed from four main places of entry, two horizonal and two vertical. The movement is at first slow and then gets more and more rapid, especially in the centre.

3. *Currents seen in the dark.* Currents can be seen in the dark which correspond in their general character to those seen in the light. The whirling in the centre is usually very noticeable. Generally pale bluish-violet circles form in the periphery and these gradually contract and advance on the centre of the field of vision. When the circle reaches the centre it breaks up into a star-shaped figure and becomes much brighter. This is then succeeded by another contracting circle.

4. *Currents seen through yellow-green glass.* I see these currents with yellow-green glass when the eye has become fatigued by looking through the glass, the whole field becomes dark and the whirling currents are seen. The general form remains the same. Fig. 7 should be compared with the drawing given by Exner[1] as the figure seen entoptically with red light. It is evident that he saw the same figure but he does not say anything about movement, this is probably due to the fact that the figure is very fugitive with red light. The position of the currents in the outer part of the field of vision seems to change continually.

5. *Currents seen with intermittent light.* If when regarding a rotating disk composed of black and white sectors we note the time when the fine flicker is most marked and keep the eyes steadily fixed on the disk, the field of vision often becomes dark red and we see a number of interlacing currents forming the figure shown in Fig. 7.

6. *Sudden cessation of currents.* The currents are usually very evanescent, but I have seen them at times continuously for several seconds. Occasionally they will all stop at once and the appearance is like Fig. 8, three cornered spots with rounded ends being seen at the junctions of the currents. These currents whilst preserving the same general form seem to change their path continually. External objects are not visible in the portion of the field of vision in which the currents are seen.

7. *Effect of the currents on an after-image.* The currents carry the visual quality, colour and brightness, of the region from whence they

[1] *Pflüger's Arch.* 1. 8. 375—391. 1868.

come into the after-image. They also tend to move the after-image towards the centre, thus if we have two similar after-images, one situated in the centre and the other a short distance from the centre, the one external to the centre may be carried into the centre and combine with the one already there.

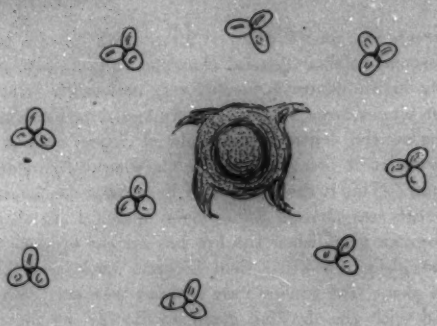


Fig. 8.

The way in which the currents encroach on an after-image may be seen in the following way. One eye being shut and covered up the other is directed to the bright blue sky whilst the open fingers are moved rapidly before it. On shutting the eye and covering both the yellow spot region is marked out as a rose coloured oval. There is a sensation of rapid and whirling movement on the outside of the oval. As the movement encroaches on the rose oval this disappears from without inwards, the last movement to be seen being the whirlpool movement like a top spinning in the centre.

8. *Effect of movement of the eyes on the currents.* The currents especially the broad ones which are found in the outer part of the field of vision are affected by movements of the eyes. It would be thought that a phenomenon so striking and so easily seen as the currents in the retina would have been observed by many, but this is not the case; from the description given by Helmholtz it is evident that he saw them when repeating Vierordt's experiment with intermittent light[1]. He differs from Vierordt in considering that the appearance is due to lymph

[1] *Physiol. Optik*, p. 533.

corpuscles in the blood instead of the circulation in the retinal vessels. After I had written this paper and especially as I had found these currents through predicting their existence I was under the impression that I was the first to state that they were not vascular phenomena, but I find that Ferree[1] has pointed this out in a very conclusive manner. He finds, and I agree with him, that the streams carry with them the visual quality, colour and brightness, of the background from which they come. Ferree considers that eye movement is the chief or sole factor in the formation and direction of these currents, but I see them quite clearly with my eye quite still and the whirling in the centre is not appreciably different when the eye is moved from one point to another. Ferree does not seem to have noticed the special relation of the movement to the yellow spot and the fovea.

IV. *Appearances due to the pigment cells of the retina.*

1. *Visibility through intermittent light.* Charpentier[2] states that if the fingers be slightly separated and then when the other eye is shut the fingers be moved rapidly backwards and forwards whilst the gaze is directed towards the blue sky the field will be covered with dark purple violet hexagonal figures, there being a light interval between each. I agree with Wolffberg that these figures correspond to the hexagonal pigment cells. They cover a much greater area of the field of vision than corresponds to the fovea and have a diameter at least four times as large as that of the circles seen in the region of the fovea.

The following experiment shows almost certainly that the appearances are due to the pigment cells; whilst my right eye was fixed on a gas flame shielded by an opal glass globe I moved from side to side in front of the eye a piece of black cardboard with a vertical slit of an inch long and a quarter of an inch wide. On moving this moderately quickly the whole gas globe appeared pure green instead of yellow and covered with hexagonal figures, each with a brighter circular spot in it. Between these hexagons were little red curved lines in which there appeared to be rapid movement. These red lines were between but did not encircle the hexagons. Increasing the rapidity of the move-

[1] *The Amer. Journ. of Psychol.* p. 484. 1908.
[2] *Compt. Rendus*, XCII. pp. 355—357.

ment of the cardboard did not alter the appearance of the phenomenon. See Fig. 9.

2. *Visibility when the retina is specially sensitive.* I can also often see hexagons of the same size on awaking in the morning but they are light with a dark interval between each.

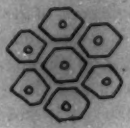


Fig. 9.

Interpretation of the phenomena.

On the theory I have put forward[1] I explain these phenomena in the following way. The currents seen are currents of visual purple flowing into the external fovea. When there is visual purple in the fovea this is the most sensitive part of the whole retina, but when there is none there time must elapse before it can diffuse into the spot.

I have three specimens prepared by C. Devereux Marshall showing the retinas of monkeys from the outer side and the arrangement of the rods and cones. The appearance of the cones of the fovea is exactly the same as the entoptic appearance of this central portion. The cones appeared as circles arranged in lines nearly at right angles to each other with a slight curve towards the centre of the fovea. I found the same with a human fovea only the cones were smaller than in the case of the monkey. On examining the external surface of the retina of a monkey there appeared four slight depressions leading to the larger depression of the external fovea. These depressions correspond to the four main branches seen in the subjective phenomena and would appear to be channels to allow of the easy flow of the visual purple. It occurred to me that if this were the case we should obtain evidence of them in cases where the outflow from the retina was obstructed as by tumour. I find that this is the case, the star-shaped

[1] *Colour Blindness and Colour Perception*, Int. Scient. Series, p. 312.

figure given by Sir Victor Horsley in his paper on tumour of the frontal lobe[1] is almost exactly the same as that seen subjectively.

All the drawings are for my right eye.

The web-like appearance seen subjectively corresponds to the cone distribution of the retina as viewed from its outer side, the portions occupied by rods appearing as dark spaces.

The yellow pigment in the yellow spot on the theory I have given should have a similar function to the yellow screen in photography.

[1] *Brit. Med. Journ.* p. 556. 1910.

THE INFLUENCE OF CARBOHYDRATE AND FAT ON PROTEIN METABOLISM. II.—THE EFFECT OF PHLORIDZIN GLYCOSURIA[1]. By E. P. CATHCART and M. ROSS TAYLOR. *University of Glasgow.*

One of us (E. P. C.) has brought forward a certain amount of evidence [1] to show that the appearance of creatine in the urine is more or less intimately connected with disturbances in the metabolism of carbohydrates in the body or at any rate with the presence or absence of these substances in the food. It was demonstrated that on a carbohydrate diet the creatine in the urine, present as the result of starvation, disappeared, whereas if the carbohydrate were replaced by fat instead of the disappearance there was a rise in the output of creatine.

These results naturally raised the following questions. When the body is deprived of its stored carbohydrate (1) is there an immediate appearance of creatine in the urine, and (2) if so, is this output of creatine associated with an increase in the output of total nitrogen?

The condition to be investigated was thus one in which the carbohydrate metabolism for some reason, artificial or other, was defective, for example some form of glycosuria. Diabetes mellitus was of no value from the experimental standpoint as (1) the glycosuria cannot be controlled at all or only with difficulty, (2) it is very difficult to control the diet of the diabetic patient both in quality and quantity of food taken, and (3) there is always some uncertainty as the true nature of the disease is so little known—whether there is only a fault of the carbohydrate metabolism or, what is much more likely in advanced cases at least, that all three metabolisms, protein, fat and carbohydrate, are affected. One of us (M. R. T.) has investigated the output of creatine in diabetes mellitus and found that this substance is constantly present in the urine[2]. Phloridzin glycosuria on the other hand seemed to offer us an ideal condition for our investigation—a simple deprivation of sugar

[1] A preliminary communication was made to the Physiological Society, March, 1910.

[2] Paper read before the Brit. Med. Assoc., London, July 29th, 1910.

without any faulty utilisation of the carbohydrate and without serious disturbance of the general metabolism[2]. (This latter statement is however only true if the supply of carbohydrate, either stored in the body or present in the food, be in sufficient amount.)

The majority of our experiments were carried out on one dog—a collie bitch weighing about 15 kilos. Between the experiments the animal had a rest of several weeks during which it was fed on an ordinary diet and had sufficient exercise. Some of the results were confirmed on another animal—a retriever bitch of about the same weight. The two sets of results were always in fair agreement. The products estimated were total nitrogen (by the Kjeldahl method), preformed and total creatinine (by the Folin method) and sugar (by the Pavy-Fehling method). The food given to the dogs during the experimental periods was creatine and creatinine free.

It will be noted that as the result of the fast, just as is the case with man, creatine appears in the urine accompanied by apparently some rise in the output of total nitrogen. On the diet given the dog lost weight although there was no appearance of creatine in the urine until glycosuria was induced. The output of sugar was also accompanied by a slight rise in the output of total nitrogen, indicating perhaps that there

Exp. I. The diet consisted of oatmeal 75 grms., milk 250 c.c., and water 500 c.c. The initial weight of the animal was 15·05 kilos, and the end weight 13·75 kilos. Previous to the experiment the animal was fasted for one day.

Day	Amount of urine passed in c.c.	Total nitrogen in grms.	Preformed creatinine in grms.	Creatine in grms.	Sugar in grms.	Remarks
1	1000	5·7	·409	·015	—	After fast
2	750	3·2	·319	·000	—	
3	700	3·0	·305	·000	—	
4	925	3·3	·339	·044	15·8	·75 grm. phloridzin
5	1050	4·2	·336	·024	1·08	
6	870	4·8	·325	·000	—	
7	1010	2·9	·337	·000	—	
8	850	2·2	·260	·000	—	

was not a sufficiency of free carbohydrate and that some of the combined carbohydrate had been drawn upon. The observation of Falta, Grote and Staehelin[3] supports such a view. These observers found, using a dog from which the pancreas had been removed, that the glycosuria was accompanied by an increase in the output of total nitrogen. They came to the conclusion that this increase was due to the demand for

carbohydrate being so great that all the direct supplies having been utilised it must now be obtained indirectly, i.e. from the protein. In our experiment with the disappearance of the sugar from the urine the creatine also disappeared, the nitrogen output however still showed a slight increase.

Exp. II. The diet was increased so that it now consisted of oatmeal 100 grms., milk 500 c.c., and water 700 c.c. The initial weight of the animal was 15·1 kilos, and the end weight 13·5 kilos.

Day	Amount of urine passed in c.c.	Total nitrogen in grms.	Preformed creatinine in grms.	Creatine in grms.	Sugar in grms.	Remarks
1	470	5·4	·482	·003	—	After fast
2	950	3·9	·391	·000	—	
3	770	3·8	·398	·000	—	
4	800	3·7	·380	·000	—	
5	800	3·2	·339	·000	—	
6	1150	4·9	·458	·030	16·1	·75 grm. phloridzin
7	860	2·9	·327	·000	—	
8	550	3·2	·304	·000	—	

Here again as the result of the injection of phloridzin there was an output of creatine which only lasted so long as the glycosuria persisted. There was perhaps also a very slight rise in the output of total nitrogen which may have been due to the demand for carbohydrate. The increased output may however in part depend on the diuresis.

In order if possible to prevent this increase in output of total nitrogen, supposing the nitrogen is excreted solely as the result of the call for carbohydrate and not as the result of a possible direct toxic action of the phloridzin, a third experiment, with a big increase of the carbohydrate intake, was carried out.

Exp. III. The diet consisted of oatmeal 100 grms., tapioca 100 grms., milk 500 c.c., and water 700 c.c. The initial weight of the animal was 15·45 kilos, and the end weight 14 kilos.

Day	Amount of urine passed in c.c.	Total nitrogen in grms.	Preformed creatinine in grms.	Creatine in grms.	Sugar in grms.	Remarks
1	355	4·9	·374	nil	—	
2	1700	5·9	·467	—	—	
3	1400	3·8	·371	—	—	
4	1350	3·1	·371	—	—	
5	1700	3·7	·432	—	27·6	·75 grm. phloridzin
6	1400	3·5	·358	—	16·4	
7	1550	5·0	·438	—	—	
8	1200	3·3	·302	—	—	
9	1400	3·7	·352	—	—	

Here it will be observed that as the result of the large intake of carbohydrate there was no rise, or at most a very slight one, in the excretion of total nitrogen. Again there was a very marked output of sugar but without any output of creatine. Therefore it may be concluded that the increase in the output of total nitrogen in the previous experiments was not due to any toxic action of the phloridzin itself but was the result of the demand for sugar from some protein source. Another experiment identical with this one on another dog gave exactly the same result.

These experiments conclusively show that (1) if the supply of carbohydrate be deficient in the organism creatine appears in the urine, and (2) if the supply of carbohydrate be abundant, in spite of glycosuria, creatine is not excreted. That the excretion of creatine is directly related in some fashion to the utilisation of carbohydrate is more than suggested by the observation that creatine is only excreted, in those experiments where the supply of carbohydrate in the food is not great, so long as sugar is being excreted. Our observations on the output of creatine as the result of injection of phloridzin have been confirmed by Krause and Cramer[4] (working independently).

The question as to whether the carbohydrate could be replaced by fat was now dealt with. We thought that by similar experiments to the above we might obtain evidence which would support the hypothesis of Chauveau that fat before utilisation is converted into sugar. The results which we have obtained up to the present do not help much towards the elucidation of this problem. Probably the explanation lies in the fact that the glycosuric episode is of much too short duration, i.e. the animal has not time to adapt its metabolism before the demand is over. Some later experiments along another line of investigation have yielded more hopeful results.

Exp. IV. The diet was made up as follows: oatmeal 100 grms., lard 100 grms., milk 500 c.c., water 250 c.c. The initial weight of the animal was 15·87 kilos, and the end weight 15·80 kilos.

Day	Amount of urine passed in c.c.	Total nitrogen in grms.	Preformed creatinine in grms.	Creatine in grms.	Sugar in grms.	Remarks
1	700	2·9	·265	nil	nil	
2	1000	4·6	·393	—	—	
3	700	3·8	·376	—	—	
4	500	2·6	·318	—	—	
5	900	3·2	·448	·028	12·6	·75 grm. phloridzin
6	500	2·1	·309	·028	2·5	
7	800	3·0	·468	—	—	
8	400	1·5	·274	—	—	
9	300	1·2	·204	—	—	

This experiment was an exact duplicate of Exp. III. except that the 100 grms. of tapioca were replaced by 100 grms. of lard, and therefore as a result the caloric value of the diet was increased by about one-half. The result however was not the same. The glycosuria was much less intense and it was accompanied by quite a marked excretion of creatine. As regards the output of total nitrogen it is difficult to decide whether it was affected or not as there were marked fluctuations in the output of the urine which of course of itself might quite easily affect the nitrogen output.

As it was possible, although hardly probable, that a still larger intake of fat might lead to results similar to those obtained with the carbohydrate another experiment was carried out.

It will be noted that it was soon evident that the amount of carbohydrate present in the diet was insufficient for the metabolic needs even before the glycosuria was induced. This is of course quite in keeping with the results obtained by one of us (E. P. C.) on man (*l.c.*). There was curiously enough as the result of the injection no rise in the output of nitrogen (further evidence that the previous increases were not due to the toxic action of the phloridzin) and no very definite increase in the output of creatine. The glycosuria was quite well marked. There was apparently little or no acidosis as the result of the fat feeding as evidenced by the absence of acetone from the urine. Krause and Cramer (*l.c.*) also observed this freedom from acidosis.

Exp. V. The diet consisted of oatmeal 50 grms., lard 150 grms., milk 500 c.c., water 250 c.c. The initial weight was 16·85 kilos, and the end weight 16·25 kilos.

Day	Amount of urine passed in c.c.	Total nitrogen in grms.	Preformed creatinine in grms.	Creatine in grms.	Sugar in grms.	Remarks
1	600	2·9	·415	·000	—	No acetone
2	500	2·5	·366	·021	—	—
3	?	·54 %	·061 %	·007 %	—	Part of urine lost
4	600	3·3	·528	·036	—	—
5	800	3·0	·568	·036	21·6	·75 grm. phloridzin
6	300	2·4	·247	·011	7·8	—
7	800	3·4	·487	·035	—	—
8	650	2·2	·436	·020	—	—

In the next experiment it was decided to increase the amount of carbohydrate present in the diet to discover, if possible, how much carbohydrate was necessary to prevent the output of creatine before the glycosuria was induced. Exp. IV. had shown that apparently 100 grms. of oatmeal were sufficient, and here 75 grms. were tried.

Exp. VI. The diet was made up as follows: oatmeal 75 grms., lard 150 grms., milk 500 c.c., water 250 c.c. The initial weight of the animal was 16 kilos, and the end weight 17 kilos.

Day	Amount of urine passed in c.c.	Total nitrogen in grms.	Preformed creatinine in grms.	Creatine in grms.	Sugar in grms.	Remarks
1	500	4·5	·284	·031	nil	No acetone
2	500	3·0	·307	·025	—	—
3	400	2·8	·396	·067	—	—
4	300	1·7	·297	·024	—	—
5	900	3·6	·611	·104	16·2	·75 grm. phloridzin
6	400	2·5	·404	·038	6·0	—
7	500	2·8	·436	·031	—	—
8	600	3·8	·406	·032	—	—

Here, as in Exp. V., creatine was steadily excreted before the glycosuria was induced, in other words 75 grms. of carbohydrate proved to be insufficient. With the induction of the glycosuria however not only was there a rise in the output of nitrogen but there was also a very decided transitory rise in the output of creatine. Again, if acetone is to be regarded as an index of acidosis none was present as the acetone tests were consistently negative.

Further it will be noted that here there was quite a definite rise in body-weight. Evidently although the fat could be stored it could not minister to the metabolic needs in the same way as carbohydrate.

It would appear then that fat in the food, even in considerable amount, does not prevent protein catabolism, in other words under the conditions and within the period of the present experiments fat cannot replace carbohydrates. It is not a question of the caloric needs of the animal being unsatisfied as the intake of calories on the fat diets was far higher than on the carbohydrate and much in excess of the animal's own requirements. It may be that fat can replace a certain amount of the carbohydrate provided of course that there still be a sufficiency of carbohydrate in the diet to carry on certain essential metabolic processes, one of which, according to Rosenfeld, is the proper combustion of fats. It is evident that the carbohydrates possess like the proteins quite a well-marked specific action.

In the remarks on the various experiments the output of creatinine was not touched on. If, however, the various tables be studied it will be observed that in every case along with the output or rise in the output of creatine there is a corresponding rise in the output of creatinine sometimes only slight but for the most part marked. This observation lends further support to the view that these two substances are, as has been

contended by many but denied by others, intimately connected. The rise in the output of creatinine takes place not only in actual amount but also, in certain experiments, as the following table demonstrates, in percentage amount. An examination of the percentage output of creatinine nitrogen does not yield a great deal except that there appears to be on the whole some slight connection between the rise in the output of total nitrogen and the rise in the output of creatinine.

Percentage of total nitrogen.

Exp.	Creatinine nitrogen	Creatine nitrogen	Exp.	Creatinine nitrogen	Creatine nitrogen
I	3·76	—	IV	4·5	—
	3·7	·5		5·2	·3
	3·0	·16		5·4	·6
	2·5	—		5·7	—
II	3·9	—	V	5·9	·4
	3·4	·3		7·0	·48
	4·1	—		3·8	·2
				4·2	·4
III	4·4	—	VI	6·4	·6
	4·8	—		6·3	1·0
	3·7	—		6·0	·5
	3·2	—		5·7	·4

In conclusion another experiment was carried out to test whether the output of creatine might not be due simply to the increased catabolism of muscle or other creatine-containing tissues as the result of acidosis. It is of course well known that acidosis can lead to an increase in the output of nitrogen. Attention has been drawn to the fact that there was no evidence of acidosis, judging at any rate by the absence of acetone, in the fat experiments already carried out.

It will be noted here that as usual there was no output of creatine during the carbohydrate period and that the output of total nitrogen was very steady. This output of total nitrogen remained steady from the commencement of the fat diet until the alkali was given. Whether the changes observed be due to the action of the alkali or merely to the alteration in the amount of urine secreted it is at present impossible to decide. On the first day of the alkali there was a sharp fall, although not by any means a very great one, followed later by a marked rise. On the day following the commencement of the fat diet the first trace of creatine appeared, the amount present rapidly increasing with, at the same time, a steady rise in the output of preformed creatinine. With

Exp. VII. The dog was fed for the first five days on a carbohydrate diet made up as follows: oatmeal 75 grms., tapioca 100 grms., milk 500 c.c., water 500 c.c., and then during the subsequent days of the experiment on a fat diet: oatmeal 75 grms., lard 100 grms., milk 500 c.c., water 500 c.c. After the output of the creatine on the fat diet was pronounced the animal was given, by means of the stomach-tube, two large doses of sodium carbonate on successive days. Throughout the fat period there was neither diacetic acid nor acetone present in the urine at any time.

Weight in kilos.	Day	Amount of urine in c.c.	Reaction (litmus)	Total acidity c.c. N/10 NaOH	Ammonia in grms.	Total nitrogen in grms.	Preformed creatinine in grms.	Creatine in grms.	Remarks
15·40	1	700	Acid	182·0	—	2·7	·457	—	Carbohydrate period
	2	1800	,,	187·2	—	3·0	·491	—	
	3	1300	,,	88·4	—	2·0	·434	—	
	4	1800	,,	98·5	—	2·8	·412	—	
	5	1400	,,	95·2	—	2·1	·365	—	
15·45	6	650	Alk. very faint	82·2	—	2·0	·339	·009	Fat period began previous day
	7	700	Slt. acid	72·8	—	2·3	·373	·058	
	8	600	Alk. faint	76·8	—	2·0	·441	·047	
	9	700	Slt. acid	100·8	·157	2·2	·465	·043	
	10	450	,,	64·6	·076	1·8	·401	·023	Na_2CO_3 20 grms. 4 p.m.
	11	1000	Alk. marked	164·0	·122	2·8	·608	·045	Na_2CO_3 15 grms. 4 p.m. Free carbonate in urine
	12	950	,,	95·0	?	1·7	·400	·014	Free carbonate in urine
	13	700	Alk.	70·0	?	1·8	·464	·015	Free carbonate (trace) in urine
15·70	14	650	Amphoter	106·6	·199	2·9	·555	?	

the administration of the alkali there was a sharp fall particularly in the output of creatine not so marked in the creatinine, followed, as in the case of the total nitrogen, by a sharp rise both of creatine and creatinine, and again followed by a fall in the output of both these substances. The fall in the case of creatine went so far that two days later it was questionable whether or no any creatine were present, on the other hand in the case of creatinine there was a steady increase in output. At the same time the total acidity of the urine (by Folin's method) and the output of ammonia (as estimated by the formol titration method) were estimated. The total acidity fell when the diet was changed from carbohydrate to fat, and throughout, even after the large doses of alkali when free carbonate was found in the urine, there was no very great variation. The ammonia was estimated from the day preceding the administration of the alkali. As the result of the first dose of carbonate there was a marked fall of some 50 per cent. in the output followed by a rise on the day following. This was succeeded by two days when no

ammonia could be detected or if present then in the merest traces, to be followed in turn by a sudden rise in the output. No full explanation of this curious rate of output can be offered.

We think however that it may be concluded from this and the previous experiments that the acidosis has little or nothing to do with bringing about the output of creatine.

Conclusions.

(1) In phloridzin glycosuria, if carbohydrate be not present in the food in sufficient amount, there is an excretion of creatine.

(2) This carbohydrate cannot be replaced by fat.

(3) The output of creatine persists so long and only so long as the glycosuria lasts.

(4) There is no apparent causal relationship between acidosis and the output of creatine.

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AN INQUIRY INTO SOME CHEMICAL FACTORS OF FATIGUE.

By W. BURRIDGE, B.A., *Dixon Scholar of Christ Church, Oxford.*

(*From the Physiological Laboratories of Oxford and Bristol.*)

THE seat of ordinary fatigue was placed by Mosso[1] in the central nervous system, for he found that a muscle, which could no longer be made voluntarily to contract, would do so on electrical stimulation of its nerve. Later investigations by Joteyko[2], however, have shown that though these nerve centres are not unfatigable, their resistance to fatigue is incomparably greater than is the case with the nerve endings.

Thus, there is a return to the older view that fatigue is peripheral, and that normal fatigue is rather a condition in which the stimuli sent out from the central nervous system, in all probability of undiminished intensity, tend to be blocked in the passage from nerve to muscle.

It is well known that certain chemical substances may cause fatigue in a nerve-muscle preparation, either by preventing the nerve impulse from reaching the muscle, a condition generally spoken of as fatigue of the nerve-ending, or by lowering the irritability of the muscle itself. The present research was carried out at the suggestion of Prof. Gotch, to whom I am indebted for much kindly help and advice, to determine further the mode of action of some of these substances.

Method. The experiments were carried out on *Rana temporaria*. The brain was first destroyed by pithing, the spinal cord being left intact. The preparation was then treated in such a way as to leave only the spinal column, back muscles, kidneys, aorta, and abdominal vein, the last being preserved with a strip of muscle on either side. The right iliac artery was now ligatured, a cannula inserted into the aorta, and the blood completely washed out of the left leg before coagulation could take place. During the breeding season the blood of male frogs coagulated so readily as to make perfusion impossible.

The preparation was fixed to the board by pins at the knee and ankle joints, and along the spinal column. A small "window" was next cut in the skin at the ankle and a thread attached to the achilles tendon.

The perfusion height was about 35 cm., and when boiled liquids were employed they were placed in flasks to avoid oxygenation.

The muscle was excited directly by needles, one placed in the region of the tendon achilles, and the other at the knee joint. Indirect stimuli were given from the cord, one needle being inserted into the spinal canal, and the other into the bone at the lower end. The current was obtained from two Daniell's cells, the secondary coils being at 0 for direct stimulation, and just supra-maximal for indirect, in the latter case the coils had no iron cores. Current reversers were placed in each circuit so that the place of stimulation was sometimes the upper and sometimes the lower end of the muscle or cord respectively. A commutator was also placed in the primary circuit, so as to send the whole of the current either to the "direct" coils, or to the "indirect," but not both simultaneously, to avoid errors due to current leakage.

The muscle was unsupported, and carried a weight of 20 grms. Break induction shocks only, at intervals of some two seconds, were used for excitation purposes, the making ones being automatically cut out by a rotating commutator.

Note. By normal saline is meant 0·6 % sodium chloride in tap water. The use of this was controlled throughout by test perfusions varying from 2 to 72 hours. In all cases both responses were found vigorous at the end of the perfusion. The substances, whose action was to be tested, were dissolved in 0·6 % sodium chloride, and then the requisite quantity of these "stock" solutions added to 0·6 % NaCl or Ringer, unless otherwise mentioned, the composition of the "Ringer" being 0·6 % NaCl, 0·025 % KCl, 0·025 % $CaCl_2$. The water used for making up these solutions was the ordinary distilled water of the laboratory, which was redistilled and condensed over glass. The action of the perfusing solutions was tested on frog's blood corpuscles to avoid errors of an osmotic type.

The action of lactic acid and lactates. The first experiments on the physiological action of lactic acid, and other substances associated with muscular fatigue, appear to have been performed by Ranke[20]. He found that the acid in small quantities increased the excitability of muscle, as estimated by the distance of the secondary coil from the primary for obtaining an induction shock which would elicit a response;

in greater concentrations it abolished the power of responding, and recovery of the muscle in these cases was found to be facilitated by the subsequent injection of weak alkalies, those actually employed being sodium carbonate and kreatinin. Later researches by the same author led him to the conclusion that his fatigue substances acted in virtue of their acidity.

More recently, Lee[6] has investigated their action and extended it to some of the lactates. Like Ranke he regards their action as being of two opposite kinds; in small quantities an augmentation of the muscle activity; in medium or large quantities, or prolonged action, a depression of activity, fatigue. The effects occurred in both curarised and non-curarised muscles, and hence their action was asserted to be on the muscle protoplasm itself.

The present experiments have been performed by perfusing different concentrations of lactic acid in Ringer's solution. As shown in Fig. 1, with small or medium concentrations of the acid (0·02—0·15 %), the series of muscular responses recorded on a slow-moving surface resembled those of an ordinary fatigue curve obtained by stimulating, now the nerve, and now the muscle, with a series of single shocks. After the primary period of augmentation the indirect response gradually diminished, and at the point of its disappearance the direct response was still vigorous. Wave-like variations were present in the later stages. On replacing the lactic acid perfusing fluid with one of normal saline alone, there was no immediate restoration of function. Instead, the muscle became œdematous, and the capability for response underwent still further diminution. With continued perfusion of normal saline this œdema gradually disappeared, and corresponding with its disappearance there was a return of response.

The magnitude of the œdema, and the length of time it lasted, and also the extent of the further diminution of muscular response, were all roughly proportional to the concentration of the acid employed.

When high concentrations of the acids were used (0·25 %) there was a rapid and complete disappearance of the indirect response, followed at a short interval by that of the direct. Perfusion of normal saline now gave an immediate and apparently complete recovery of the direct response, but none of the indirect. In a very short time, however, the perfusion of normal saline being continued, the direct response began to decrease in magnitude, whether the excitation was continuous or not, until in from 4 to 6 hours it became imperceptible. At this stage the œdema of the muscles was very marked, and in some cases the

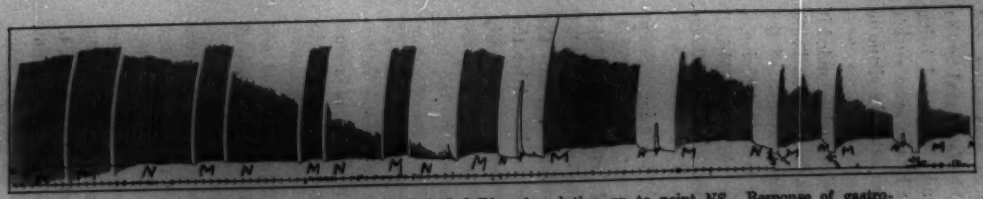

Fig. 1. Perfusion of 0·017 % lactic acid in boiled Ringer's solution up to point *NS*. Response of gastrocnemius to groups of induction shocks applied directly and indirectly. *N* = indirect excitation. *M* = direct excitation. *T* = a series of make and break shocks in quick succession (tetanus). Perfusion of normal saline was commenced at point *NS*. 5 m., 15 m. and ½ hr. represent intervals of 5 minutes, 15 minutes and ½ hour respectively. Time marked every 10 seconds.

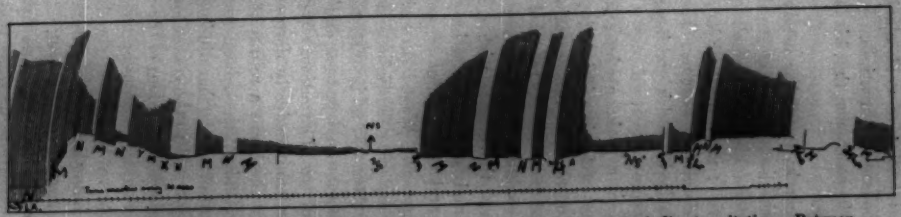

Fig. 2. Perfusion of 0·25 % lactic acid in 0·6 % NaCl. *M* = direct excitation. *N* = indirect excitation. Between *Y* and *X* the perfusion was stopped resulting in some degree of spontaneous recovery. *LA* marks commencement of perfusion of the acid. *NS* marks commencement of perfusion of normal saline. At 2 m., 5 m. and ½ hr. there were intervals of 2 minutes, 5 minutes and ½ hour respectively. 4 hrs. and 24 hrs. represent times that had elapsed since the commencement of the experiment.

perfusion was stopped here: the muscles were dissected out, weighed and their weights compared with those of the opposite control legs. There was a distinct increase in weight amounting to 30 %.

In those cases where the perfusion of saline was continued further (50—90 hours), some recovery both of the direct and indirect responses was noted, but with marked differences in their extent. The former would attain a value comparable with its original magnitude, but the latter in the most favourable cases would never attain more than half that of the other, the direct; usually it was much less as may be seen from Fig. 3.

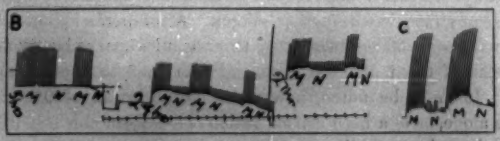

Fig. 3. *B* and *C*=stages in the recovery of a muscle after a second perfusion of 0·25 % lactic acid in Ringer. The times marked on tracings represent times that have elapsed since experiment began. *M*=direct excitation. *N*=indirect excitation. Time marked every 10 seconds. In *C* the tall and the short are the responses given to the direct and indirect excitations respectively after 56 hours' perfusion with normal saline.

Although the magnitude of response returned to its original value almost immediately on washing out the lactic acid solution with normal saline, the effects noted with further perfusion seemed to show that the recovery of the muscle itself was not complete. This could be further demonstrated by perfusing with lactic acid a second time immediately after the first recovery. The rate of failure was found to be so greatly accelerated as to be almost immediate, and the subsequent apparent recovery with saline was both retarded and incomplete. Œdema effects then occurred as before. The general results of perfusion with these high concentrations are shown in Fig. 2.

Fibrillary contractions were seen when medium and high concentrations of lactic acid were used, and there was also a distinct shortening of the muscle, which latter also occurred during the period of œdema.

The results noted were unaffected by oxygenating the perfusing liquid containing the acid, by first boiling and then passing carbon dioxide through, or by the presence or absence of calcium salts.

It was also found possible to obtain a vigorous indirect response from a muscle the nerve of which had been placed for 48 hours in lactic acid of the highest concentration (0·25 %) used in these experiments.

Sodium lactate had no observable effect on the direct response at low and medium concentrations (0·08—0·04 %), but caused the indirect to diminish gradually. This latter depressant effect occurred in a somewhat anomalous manner. The diminution could be observed throughout, but it was in great part one of diminution to excitation by regular single break shocks; on throwing in the make shock as well as the break, the muscle would respond to these make shocks to the same extent as it did before to the break, but the response now to the break was greatly increased, and reached a value comparable to that of the direct, at any rate, in the earlier stages. After perfusion had been in progress for some 30 minutes or so, this second response became smaller, while that of the make was zero, and also that to the simple break shocks. It must be noted, however, that perfusion of 0·6 % sodium chloride alone, or of a high concentration (1—2 %) of this salt in Ringer, was found to give a similar result.

When high concentrations of sodium lactate were employed, both responses rapidly became smaller, and also the time of the whole process of contraction became increased. Complete recovery took place on subsequent perfusion of normal saline.

The above results were such as were obtained with the chemically pure sodium lactate of Merck. Experiments were performed with two other specimens of the 'pure' commercial sodium lactate. Their action was found to be rather that of a poison. Both responses gradually failed and the whole time of contraction also increased. No recovery took place when normal saline was substituted for the lactate, except where a high concentration had been employed, and in those cases the actual amount of recovery was small.

Potassium lactate. Perfusion with this salt gave a primary augmentation of both direct and indirect responses followed by depression, this latter affecting the indirect much more than the direct. The lower concentrations employed would do away with the former while leaving the latter comparatively unaffected. As the concentration was increased, the direct became more and more influenced until it, too, would rapidly vanish. Perfusion of normal saline was then followed by a rapid return of the direct but little or none of the indirect; the type of tracing obtained at this stage was very much like those obtained under similar conditions with lactic acid. No œdema was observed, however, and

complete recovery of the indirect response occurred in about an hour or so.

Ammonium lactate also influenced the indirect response more than the direct, there being, at low or moderate concentrations, little or no observable effect on the latter beyond wave-like variations when the former had vanished. In one experiment, a stray bubble of air in the cannula accidentally stopped perfusion. This occurred at a stage when the indirect response had been reduced to about one-fifth of its original value. A spontaneous recovery then began, which only ceased when the cause of obstruction had been removed and perfusion had been re-established. Artificial blocking of the perfusion by placing a clip on the rubber tubing connecting the cannula with the reservoir of perfusion liquid gave similar results, both as regards the effects of stopping the perfusion and its re-establishment. The conclusion was drawn that the muscle contained within itself the means for disposing of ammonium lactate in such quantities as had been perfused.

With high concentrations of the salt both responses rapidly disappeared, and also rapidly returned on subsequent perfusion of saline though with some lag of the indirect. In general, the muscle made a good recovery from ammonium lactate.

Calcium lactate gave some slight primary augmentation, but at medium and low concentrations there was very little other effect observable. After one hour's perfusion of 0·1—0·4 % of the salt in Ringer solution, the direct response was apparently unaffected, and only a very small decrease noted in the indirect. Both responses disappeared when high concentrations of the salts were employed (up to 3 %). Subsequent recovery with saline was good, though that of the indirect took distinctly longer than was the case with the direct response.

It will be noted that the action found for each lactate at low and medium concentrations, by which is meant for the lactates those concentrations at which there was apparently no osmotic factor, bears a marked resemblance to that found for the positive ion by other observers, except in the case of the sodium ion. This last is not usually regarded as actively affecting the indirect response, though there is evidence available which suggests that such may be the case. Thus Locke[6] found a muscle placed in 0·6 % sodium chloride quickly lost its excitability to indirect shocks, whereas the direct excitability persisted for some 24 hours. The addition of 0·2 % calcium chloride restored the indirect response in five minutes; moreover, perfusion

of 0·6 % sodium chloride has been shown by Harvey Cushing[46] to result in a rapid failure of the indirect response followed by an even quicker recovery on subsequent perfusion of a physiologically balanced solution. Lithium, ammonium and potassium have also been shown by Overton to act on the indirect response in what he terms a 'curarising' manner, and as sodium is a member of the same chemical family it does not seem improbable that it should possess the similar property of actively abolishing the response from the nerve. The results given above with respect to the perfusion of high concentrations of sodium chloride seem also to support such a view.

The lactate ion, however, appears to possess only slight fatiguing properties, if any, on the direct response, though it has a much more marked effect on the indirect. Thus all the lactates give some decrease in the indirect response on perfusion, though this effect was somewhat masked with the calcium salt. There is also the lag of recovery of the indirect when high concentrations of the salts were used, and such lag was found to be distinctly greater than was the case with perfusion of the corresponding chlorides. To examine these points further a solution was made up containing the same weights of sodium, potassium, and calcium as are present in an ordinary Ringer's solution, but with lactates substituted for chlorides. This solution was then perfused. At first the muscles of the experimental leg entered into a state of fibrillary contraction, which later subsided. No other effect was noted for some time save a depression in the amount of the indirect response. At the end of two hours the direct preserved its original height, but the total duration of each twitch (contraction and relaxation) had been so greatly increased that it was possible to produce a condition of tetanus with stimuli at half-second intervals. The indirect response had by this time disappeared or nearly so; later the direct response also decreased in height, and at the end of some 8 to 10 hours had vanished, but the muscles still gave a slight response to a tetanic series of strong shocks. The conclusion was then drawn that the lactates had a fatiguing action on the direct response, though very small, and a more marked effect on the indirect response. The properties of such a perfusing fluid are receiving further attention, since considering the position of lactic acid in metabolism, it is conceivable that the inorganic lactates may have an energy value.

As already mentioned, each of the lactates at high concentrations had an apparent fatiguing action in that the responses rapidly disappeared and subsequently recovered on perfusion of saline. Examination

of such solutions showed that they were hypertonic. Accordingly, experiments were performed in order to ascertain what possible effects were produced by these means, and the method adopted was the perfusion of concentrated solutions of the chlorides of sodium, potassium, and calcium, the actual strengths employed varying from 1 to 5 % in distilled water. A hypertonic solution of sodium chloride gradually abolished both responses with the more marked effect on the indirect as mentioned before. As the direct response diminished so the total time of contraction increased, the curves resembling those of a fatigued muscle. Some shortening of the muscle also occurred. Recovery took place on subsequent perfusion of saline. Potassium chloride rapidly abolished both responses, which just as rapidly recovered on subsequent saline perfusion, provided this had taken place immediately after or before the failure of response was complete. If, however, the hypertonic solution was allowed to perfuse for some time after this complete failure had occurred, the subsequent recovery with normal saline was delayed and also there was a marked lag in that of the indirect response. The action on the indirect response, however, was never found to be so great as that which occurred with corresponding concentrations of the lactate. Other effects of these high concentrations of potassium chloride will be described later. Calcium chloride also rapidly abolished both responses. There was a very slight shortening of the muscle at first which was followed by an elongation. The recovery with saline was good, and, though a delay was present in the indirect response, it was not so prolonged as with the lactate. No signs of a 'drawing out' of the contractile process were observed with calcium or potassium. The results thus obtained showed that the sodium and potassium ion, when combined with another innocuous ion, could give effects simulating fatigue provided suitable concentrations were employed, those actually required being a hypertonic one of the sodium salt and a fairly low concentration of the potassium one; so that statements regarding the fatiguing action of salts of these metals must be accepted with some caution, unless the actual concentrations employed are also given.

All the lactates perfused readily, the rate being from two to four times as great as that of a solution of normal saline at the same height, suggesting that the lactates may have a specific action in dilating the arterioles of a muscle.

As regards the action of lactic acid, an examination of my records shows that it has the augmenting and fatiguing properties discovered by Ranke[10]. The effects noted, however, on subsequent perfusion of

saline seem to show that in addition an internal (intrafusal), and an external (circulatory) action must be assumed. Both of these produce a fatiguing effect, but that due to the intrafusal cause is much longer lasting; for whereas the circulatory may be got rid of by washing out the muscle, removal of the intrafusal would appear to depend on some form of oxidation such as that proved to occur by Hopkins and Fletcher[7]. It may be fairly assumed that this internal lactic acid must be the greatest at the time of commencement of perfusing normal saline. In spite of this, the muscle response makes a full recovery at first, while the subsequent diminution that occurs varies with the extent of the œdema. Thus it appears that the œdema, qua œdema, is the cause of this subsequent diminution, possibly as suggested by Ranke[8], acting as a diluent of the contracting materials; though there is no doubt that lactic acid is the cause of the œdema. The dependence of the contractile process upon a sufficient increase of osmotic pressure in the muscle fibre has been pointed out by Macdonald[9], and the presence of this œdema would undoubtedly cause a lessening in the extent of the osmotic pressure by dilution, and so make the contraction less efficient.

There is also the possibility that intrafusal lactic acid of itself is not fatiguing. On chemical grounds a fatigue substance should represent an end product of the chemical action leading to contraction. The high energy value of lactic acid seems to negative such a conclusion, and the acid maximum of tetanus is much less than the acid maximum of extra-physiological processes, making it possible that though the process of contraction has been brought to a close by fatigue, those leading to the production of lactic acid have not. But it should be remembered in this connection, that the method used by Hopkins and Fletcher[7] to fatigue a muscle is not a complete one; for it has been shown by Joteyko[10] that when a muscle no longer replies to induced currents, it is possible by means of a galvanic current to produce a fresh series of long-continued contractions, which also undergoes a fatigue process, and it is probable that the acid maximum of fatigue can be brought nearer to the other maxima.

Comparing the results obtained with lactic acid and the lactates, it can be seen that most of the effects produced by the former are due to the action of the hydrogen ion. The lactate part has comparatively little action, even on the more susceptible indirect response, but the subsequent œdema after perfusion with saline and also the harmful effect on the indirect response would appear to be wholly due to the

hydrogen ion. Vernon[9] has shown in another connection, that the action of lactic acid was in accordance with the extent of its ionisation.

Potassium chloride. Potassium salts have been shown by Overton[10] to abolish the indirect response of muscle. This action can be shown by perfusing a comparatively weak concentration (0·05 %) of potassium chloride in Ringer. The indirect response could be abolished when the direct was still at its full strength. On perfusion with normal saline recovery was complete and fairly quick. Both responses were affected on increasing the amount of potassium salts, but the indirect much more than the direct as was also shown by the lag of recovery. After several successive perfusions of potassium, followed by washings out with normal saline, this lag of recovery of the indirect was so increased as to leave the direct response at its original value when the indirect had only recovered to a very slight extent.

A still further increase in the amount of potassium accelerates the rate of failure of both responses, and also diminishes the amount of lag in the recovery of the indirect response on a first washing out with normal saline, although successive perfusions will make the lag manifest. Fibrillary contractions occurred in the muscles when these increased amounts of potassium were perfused.

It was noted in addition that potassium chloride had a primary augmenting action on both responses, thus confirming Mines[11] in the case of the direct, and that the above results were not affected by the presence of the calcium salts in the Ringer solution, as also shown by Overton[10], by lack of oxygen, or by excess of carbon dioxide.

It was also considered of interest to examine the character of the contractions given by a muscle as it gradually failed under the action of potassium chloride. This was done on a fast moving recording surface. The total time of the whole process was found to be practically unaltered, the muscle curves merely becoming less steep, and developing a slight plateau on the top. The greatest concentration of potassium chloride used in any of the above experiments was 0·225 % KCl (0·2 % added to 'Ringer').

In the next group of experiments high concentrations of potassium chloride were perfused (1·5 to 5 %, in distilled water). Such concentrations were shown by Grützner[12] to produce a slow and long continued contraction in muscle, his results being confirmed by Zenneck[13] and Zoetout[14]. The last found the contraction to persist for times varying from 20 to 100 minutes.

In my experiments when the perfusion pressure was at its usual height (about 30 cm.) the contraction of the arteries[10] and muscles in the thigh region was sufficient to prevent for a time the access of fluid to the lower parts, but when some slight relaxation had occurred, the liquid apparently passed through slowly to the gastrocnemius, which then entered into contraction in a series of steps due presumably to the different times at which different sets of fibres supplied by each artery became affected. By raising the vessel containing the perfusing liquid to a height of some 70 cm., and also by suitably pinching the rubber tubing connecting this to the cannula, it was possible to perfuse, or rather inject, all parts of the leg practically simultaneously. Under these conditions the muscle rapidly shortened, remained in the fully contracted state a short time (15. to 30 secs.), and then gradually relaxed, returning in 5 mins. to about half its original length. Probably then the very long time of contraction noted in some of Zoetout's experiments is due to the use of baths, for then the fibres in the centre of the muscle would not become affected until diffusion had taken place from the outside. The height of the contraction was always greater than that produced by a single maximal induction shock, and for these and other reasons, I regard it as tetanic in character.

Fig. 4. Effect of perfusion of 0·04 % acid sodium phosphate in 0·06 % sodium chloride. *M* = direct excitation. *N* = indirect excitation. At *NS* perfusion of normal saline was begun, and the amount of recovery after ½ hour is also shown.

Acid phosphates. A solution of acid sodium phosphate in 0·6 % NaCl was associated with a rapid failure of the indirect response, this falling to zero while the direct was still intact. The phosphate was employed at various contractions, and in all cases the direct was still vigorous at the time the indirect had vanished. The amount of primary augmentation given by acid sodium phosphate, if any at all, is exceedingly small.

Recovery of the indirect response by perfusion of normal saline was complete at all concentrations, though it took some three or four hours.

Addition of an acid phosphate to normal saline caused the formation of a precipitate which could, however, be dissolved by passing CO_2 through the liquid. Such a solution had a less marked effect on the indirect response, but the failure of this before the direct was still present. In this connection, the remarks regarding the effect of perfusing a liquid with insufficient calcium, as mentioned under the lactates, is of importance.

I found that when a small quantity of potassium chloride was added to the acid sodium phosphate solution, a distinct augmenting action was seen both on the direct and indirect responses. There were marked

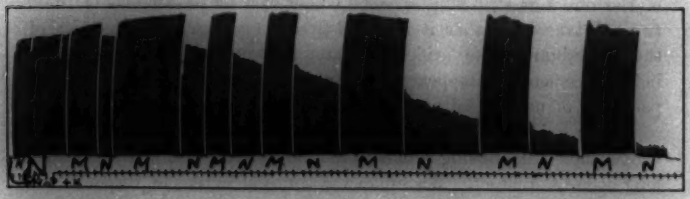

Fig. 5. Same strength of acid sodium phosphate as employed in above expt. (Fig. 4) but with the addition of 0·04 % potassium chloride. *M* = direct excitation. *N* = indirect excitation.

differences as regards the length of time during which this augmentation persisted. That of the indirect was much briefer in duration, and depression occurred while the direct still increased its vigour. The nature of the action may be understood by reference to Fig. 5. There is a complete though somewhat delayed recovery of both responses to perfusion by normal saline.

Acid potassium phosphate acted similarly to the sodium compound when potassium salts are also present in small amounts. Recovery was complete. In regard to this Overton[30] has also found that acid phosphate is without permanent injurious effect on the nerve endings.

Wave-like variations were observed in the fatigue curves obtained with acid phosphates, with a solution of carbon dioxide in Ringer, with ammonium lactate and as mentioned above with lactic acid. The

physical conditions of the production of these waves have been investigated by Symons[18], who also noted an earlier production with lactic acid and acid potassium phosphate. Though the circumstances of excitation throughout were similar, I have not noted the production of these waves when neutral salts, such as potassium chloride, sodium lactate, etc., were perfused. All the substances with which they were produced, with the exception of ammonium lactate, contain the hydrogen ion, leading to the conclusion that the stage at which these waves may be produced is one at which a free acid is in process of neutralisation by ammonia.

Potassium chloride and an acid phosphate. As already mentioned, the addition of a potassium salt to an acid phosphate caused an augmentation of both responses, that of the indirect being much shorter in duration and changing to depression while the augmentation of the direct is still in progress. Under varying proportions of these two substances together it is found that the greater the proportion of the potassium to the acid phosphate, the quicker both direct and indirect responses disappeared, and also the quicker the recovery on perfusion of normal saline.

Lactic acid and acid phosphate. Mixtures of these in 0·6 NaCl or Ringer have a depressing action on the indirect response which vanishes long before the direct. Wave-like variations of extent of response are usually present in the later stages. Recovery on perfusion with normal saline is slow but complete. When calcium salts are present (Ringer) the indirect response takes longer to disappear, but otherwise the results are the same.

A solution of carbon-dioxide in 'Ringer,' with or without lack of oxygen, was found to do away with the indirect response at a time when the direct was apparently unaffected. Wave-like variations of response were noted in the tracings obtained.

Potassium chloride, lactic acid, etc. These two substances together have a marked action on the indirect response which is shown by its rapid disappearance as compared with the direct, and also by its slower rate of recovery. Here, as with the acid phosphate, the rate of recovery on perfusion with normal saline depends on the magnitude of the ratio between the amount of potassium salts present and that of the lactic acid.

It should be noted that the above results were obtained when the supply of oxygen was fairly good, the perfusing solutions being shaken with air. After a perfusion which has allowed of a complete recovery,

a repetition of the whole process will bring out a marked lag of the indirect response.

If, however, the solution of potassium chloride and lactic acid be first boiled and then perfused, lag of the indirect recovery on perfusion of normal saline will immediately become apparent. The passage of CO_2 through the mixture after boiling further increases this lag. The type of tracing thus obtained is shown in Fig. 6.

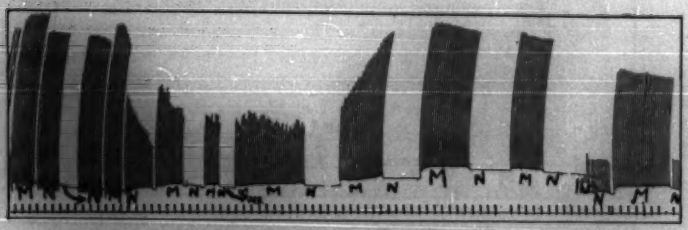

Fig. 6. The perfusing solution was 0·6 % sodium chloride, 0·22 % potassium chloride, 0·024 % calcium chloride, 0·01 % lactic acid. This was first boiled and then carbon dioxide bubbled through during the time it cooled. The first arrow marks the point where perfusion of this solution was commenced and the second arrow (*NS*) the commencement of perfusion of normal saline. *M* = direct excitation. *N* = indirect excitation. 1 hr. = interval of one hour. Time marked every 10 seconds.

With regard to such perfusing mixtures, it may be generally stated that a solution, which in presence of oxygen allows of a complete recovery of the indirect response on perfusion with normal saline, will in absence of oxygen and presence of CO_2 abolish the recovery for some time. Calcium salts and oxygen are necessary for this recovery.

The addition of an acid phosphate to the perfusing mixture assists recovery, and a solution which, as above, entirely abolished the indirect response for some time, will on adding sufficient acid phosphate allow of a complete recovery with normal saline.

Further, a solution of the three substances in which the phosphates are not present in sufficient amount, will by adding kreatinin or kreatin before boiling allow of a good recovery. Kreatin added after boiling is not so efficient as before boiling.

The amount of lactic acid required to produce these results is small, the maximum ever employed in these experiments being 0·01 grm. in 100 c.c. of liquid. It varies according to the season of the year,

the summer frog being much less resistant than the autumn and winter.

Solutions of these three substances have been obtained which immediately abolished the indirect but not the direct. The amounts, however, exhibit great variations and appear to vary with each individual and the season of the year.

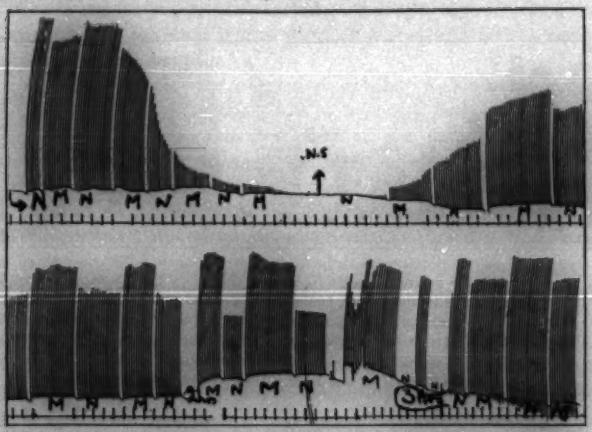

Fig. 7. The perfusing solution 0·6 %, sodium chloride, 0·22 % potassium chloride, 0·01 % lactic acid, 0·023 % calcium chloride, 0·0122 % acid sodium phosphate, 0·002 gm. kreatin. Mixture was boiled and carbon dioxide passed during cooling. Lettering etc. same as before. The tracing shows after effects followed by complete recovery.

As already mentioned under lactic acid, the perfusion of saline was followed by a diminution of response with subsequent recovery. A similar action was noted when the above mixtures containing lactic acid were perfused, but in these cases it was almost confined to the indirect response, probably on account of the small quantities of acid used, which as above mentioned never exceeded 0·01 %. The recovery of the indirect might be equal to that of the direct during the early stages of the saline perfusion, but then began to fall away while the direct increased further. The effect occurred whether excitation was continuous or not, and it was not therefore due to fatigue consequent on excitation.

It appeared to depend to some extent on the rapidity of perfusion of the washing-out solution, for a series of wave-like variations with large amplitude could be produced in the indirect response by alternately raising and lowering the perfusion pressure, the upward movement following the rise of pressure and the downward the fall. The general tendency was, however, to fall, each successive wave being shorter than the preceding one, neither was it found possible to prevent this subsequent diminution by keeping the saline perfusion at a high pressure throughout. No such diminution was ever observed with the direct response though waves of variation were usually to be noted, suggesting the presence of the hydrogen ion.

Thus we have still further evidence pointing to the fact, as mentioned before, that lactic acid in virtue of its hydrogen ion has a very marked depressing influence on the indirect response. It also appears probable that lactic acid cannot exist free and uncombined in the muscle fibre; for though it appears to be an easy matter for the acid to pass from the perfusing fluid into the muscle substance, the œdema effects already noted seem to suggest that its subsequent removal may depend wholly or in part upon oxidation, and not by diffusion which latter would be the case if it were free.

The recovery of a muscle after tetanus. Ranke[3] noticed that a frog electrically tetanised to exhaustion recovered on washing out the blood with salt solution. He did not, however, differentiate between the direct and indirect responses.

Experiments have been performed with a view to elucidating what differences might be present. It was found that muscles which had been directly tetanised until they gave no direct response rapidly recovered their power of giving such response on washing out with normal saline, but would give very little indirect response. The magnitude of the direct response underwent some diminution as perfusion progressed, and then began to increase once more; the diminution being accompanied by œdema and the increase by its disappearance. Nothing other than a partial recovery of the indirect response was ever obtained when the muscle had been tetanised to such an extent as to lose its direct response (48 hours after).

Wave-like variations of response were noted throughout the œdema period following the perfusion of saline; such waves occurred under similar conditions after lactic acid perfusion.

When, however, tetanus had only been prolonged sufficiently to produce some diminution in the amount of the indirect response

(one-third of original value) perfusion of normal saline caused an immediate and complete return of this to its former value, and spontaneous recovery was also possible at this stage. If tetanus had proceeded much further, recovery with normal saline was a very slow process occupying some hours, and was of such a character as to allow of the general statement that the greater the diminution of response by tetanus, the longer the time taken proportionally for recovery, the relation being far from a linear one. Such a tardy and a quick recovery may be noted on reference to Fig. 8. The recovery of the indirect response under these circumstances exhibited no variations whether tetanus was due to direct excitation, or to stimulation of the cord or of the nerve trunk. It was also noted that a slow recovery of the indirect response occurred always at those stages where wave-like variations showed their appearance in the curves.

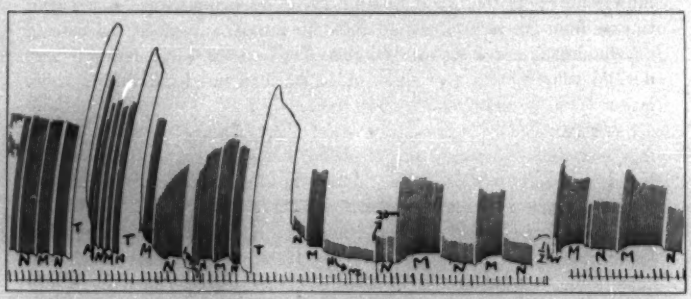

Fig. 8. Recovery of the indirect response after different periods of excitation. *T* = commencement of tetanus. The perfusion of normal saline was stopped immediately before tetanising and resumed immediately it was over except where otherwise stated.

Concluding Remarks.

Ability to recover is a salient feature of normal fatigue, but the rate of recovery has been found by Mosso[1] and his pupils to depend on the extent of the preceding fatigue. In the experiments of Maggiora[1] it was shown that a muscle worked at a rapid rhythm to complete exhaustion required a long time to recover completely (one to two hours).

It was also shown that the last contractions of a series were the most exhausting, since if only the first part were carried out the muscle took a much shorter time proportionately to recover. The same effect has been shown above to occur in the stages of recovery of a muscle from tetanus; for whereas when the indirect response had only slightly diminished the time taken for recovery was a matter of seconds, recovery after complete exhaustion did not occur within the remaining life of the muscle.

Turning to the results obtained with possible fatigue substances, it has been shown that a quick recovery is to be associated with potassium salts alone or in excess, while a slow and prolonged one has only been obtained as an effect of lactic acid. It has also been shown that the effect of an exhausting tetanus is almost identically the same as that found to occur when a high concentration of lactic acid has been perfused through a muscle. There is thus reason to believe that the results noted in the early stages of fatigue are mainly referable to potassium and those in the later stages to lactic acid.

The effect of potassium and lactic acid together appears to be a twofold one, first that of increasing the susceptibility of the indirect response to the action of the acid, and secondly, to increase its powers of recovery.

The actual conditions present in a muscle working at a fairly high rate are the continued liberation of high concentrations of potassium ions[15], production of lactic acid[6], together with a general lack of oxygen[14] and increase of tension of carbon dioxide. The small quantities of lactic acid required to paralyse the nerve-ending for some time under such conditions—never more than 0·01 % (in the demonstration given to the Physiological Society at Oxford in June, 1909 with the summer frog, only 0·003 %)—negative, I think, any view that this lactic acid can remain unneutralised, for Hopkins and Fletcher[7] found such amounts in "resting" muscle. In this connection attention may be drawn to the possible rôle of kreatin and kreatinin, both without effect on the muscle substance, and kreatin stated to have favourable influence on nerve ending, and they were both found capable of neutralising this lactic acid. The change from kreatin to kreatinin and vice versa is accomplished with ease under physiological conditions and the effects noted above give more than sufficient reason for the presence of a strong base in a muscle which seemed so improbable to Mellanby[16]. The total amounts of kreatin and kreatinin in a muscle are, however, little altered as a result of tetanus, a slight increase in the excised

muscle[17], and a slight decrease when the circulation is intact[18]. I found that it was possible to demonstrate small amounts of kreatinin in the perfusion liquid coming from a leg which was continually tetanised for some twenty-four hours. Mellanby[16] has also pointed out (as did Ranke[8]), that the amounts of kreatin found in muscles seemed to bear some relation to their functional activity. I would accordingly suggest that each muscle contains within itself such a quantity of kreatin as is sufficient to neutralise by the change to kreatinin the amounts of lactic acid which may be regarded as produced during its accustomed activity.

Should a muscle be put to an unusual stress it will exceed the kreatinin neutralisation limit, and considerations of general metabolism suggest that in such cases ammonium lactate will be formed. This has been shown above to possess the property of blocking the impulse from the nerve into the muscle, and some evidence was given that the muscle contained within itself the means for disposing of it. There is abundant evidence available for believing that this disposal is accomplished by the formation of purins. Moreover, it is in overworked muscles that this purin formation takes place[19], the amount formed gradually decreasing on successive days as the muscles become accustomed to this extra amount of exercise. I would suggest further then that part at least of the physiological processes involved in the training of athletes is an increase in the total kreatin content of their muscles, to meet the extra production of lactic acid that occurs when the muscles become harder worked.

It has already been mentioned that the effects of fatigue are mainly referable in the early stages to potassium salts, and in the later to lactic acid. The characters of a fatigue curve taken on a fast-moving drum are well known, and an examination of these will lead to the conclusion that in the later stages the condition of the muscle is more one of inability to relax than of inability to contract. This may become so marked in the intact muscle that when greatly fatigued it cannot relax but enters in to 'contracture.'

A muscle perfused with lactic acid gives duration curves similar to the ordinary fatigue curves, and also gives a distinct diminution of height of contraction. On the other hand, potassium salts do not appear to affect the time of contraction at all. This last would appear to be probable when the work of Macdonald[20] is considered. For if a muscular contraction is the result of a sudden liberation of potassium salts formerly bound up in a colloid, the presence of free potassium salts

might reasonably be expected to limit this liberation, and impose no delay as regards the return. Lactic acid, however, does impose a delay in this return, and considering that the hydrogen and potassium ions carry like charges but at different potentials, it seems probable that in these late stages there is an adsorption compound formed between the lactic acid and the colloid with which the potassium is normally adsorbed, the hydrogen ion being one of greater potency in this connection. The œdema observed with fatigue, and lactic acid, are thus referable to free displaced potassium salts which would remain free until the lactic acid had been oxidised. The fluid from preparations which had been tetanised and perfused for some twenty-four hours was found to show a notable increase in the amount of its potassium salts, and it seems highly probable that these have their origin in the manner suggested above. Moreover, we may conclude that such potassium salts in the circulation give rise to the general fatigue, both mental and physical, which follows the performance of heavy muscular work. For Mosso[21] has demonstrated that the blood of a fatigued animal is fatiguing to another, and that the cause is not referable to such substances as lactic acid, etc., inasmuch as the blood remains alkaline; while Abelous[22] has shown that its action is more particularly on the nerve-endings.

Finally, I would suggest that the fatigue toxin and anti-toxin described by Weichardt[23] are to be regarded as having a twofold nature. There are primarily the very small amounts of free lactic acid which a muscle is able to produce under the influence of stimuli sent out from the central nervous system, which could be oxidised by the oxygen (anti-toxin) of the blood corpuscles; and secondly, some product of muscular disintegration due to autolysis of a markedly acid muscle on standing, which being of protein nature would give rise to an anti-body on injection.

Summary.

1. The motor nerve-endings of a muscle were found to be more susceptible to the action of each of the possible fatigue substances examined, than was the case with any of the other elements in a muscle and nerve preparation.

2. Potassium salts have a primary augmenting action on both the direct, as shown by Mines, and indirect response of a muscle, and this augmentation increases with increase of concentration of the salts until actual excitation takes place. Solutions of pure potassium chloride

1·5 to 5% in distilled water were found capable of evoking a contraction and tetanus in a muscle independent of any other mode of excitation.

3. At low concentrations potassium salts have in addition a fatiguing action. This latter corresponded to normal physiological fatigue in affecting primarily the nerve-ending, and in its quick and easy possibility of recovery without any observable after-effect.

4. The action of potassium salts never appeared to be poisonous, for a complete recovery of a muscle and nerve was obtained after perfusion of all concentrations of the salt.

5. Evidence has been brought forward for believing that free circulating potassium salts, having their origin in working muscles, form an important factor in the general fatigue observable after heavy work.

6. The action of lactic acid corresponded closely with that due to the excessive fatigue of an overworked muscle. The nerve-endings were very markedly affected, and their subsequent recovery from the effects of the acid took some time. The effects produced are almost entirely due to the hydrogen ion.

7. Under the conditions assumed to exist in a hardworking muscle even such traces of lactic acid as have been found in 'resting' muscle, were found capable of abolishing the indirect response for some time. Hence it was considered that lactic acid must be neutralised, and that the limit of the working capacity of the intact muscle is reached at the stage when lactic acid has been produced so as to be present in the free condition beyond the merest traces. When such free lactic acid is present, the subsequent recovery of the indirect response will show a more or less marked delay, e.g. stiffness.

8. A possible rôle of kreatin is the neutralisation of lactic acid; and where this is insufficient, ammonium lactate, with subsequent formation of purins, may represent a further attempt at such neutralisation.

9. The hydrogen ion is a necessary concomitant of waves of variation in the response of a fatigued muscle.

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THE INFLUENCE OF THE PRESENCE AND POSITION OF THE VARIOUS RADICLES OF ADRENALIN ON ITS PHYSIOLOGICAL ACTIVITY[1]. By C. H. H. HAROLD, M. NIERENSTEIN and H. E. ROAF.

(*From the Physiology Laboratory, University of Liverpool, and the Chemical Department, University of Bristol.*)

It is important to discover what groups or groupings in the molecule are responsible for the physiological action of drugs of known composition. In the case of adrenalin a certain amount of work has already been done.

Dakin[8], as the result of experiments on the physiological action of substances allied to adrenalin, came to the following conclusions. (1) The catechol nucleus is necessary to produce substances like adrenalin. (2) Both hydrogens of the hydroxyls must remain unsubstituted. (3) An alkyl group of low molecular weight attached to the imide group gives a much more active substance than when an aromatic group is attached, whilst derivatives of piperidine, heptylamine and benylamine occupy an intermediate position. (4) When a simple aliphatic group is present, reduction of a ketonic base enormously increases the physiological action. (5) There appears to be a connection between chemical instability and physiological activity.

Loewi and Meyer[11] also state that the reduction products of the ketonic bases are more active than the ketones themselves.

It has been shown by Cushny[4] and confirmed by Abderhalden and F. Müller[3] that of the two isomeric reduction products of the ketone corresponding to adrenalin, it is the lævo-rotatory compound that is active and that corresponds to the natural substance obtained from the suprarenal glands.

[1] The expenses of this research have been defrayed by a Grant from the Government Grant Committee of the Royal Society.

In the present paper are recorded the results of a number of experiments performed with substances containing groups similar to those found in adrenalin.

Series 1: *Substances without an Amine Group.* Dakin[5] found that catechol caused a rise of blood pressure when injected into the circulation, so the following series of compounds were tested. Benzoic acid, mandelic acid, phenol, *o*-, *m*-, and *p*-oxybenzoic acids, resorcinol, hydroquinone, catechol, protocatechuic aldehyde, protocatechuic acid, β-resorcylic acid, phluroglucinol, oxyhydroquinone, and pyrogallol. Unfortunately we were not able to prepare *o*-oxymandelic acids but Ellinger and Kotake[7] have recently synthesised the compound.

Series 2: *Substances containing an Amine Group.* As the preceding series gave comparatively little result in the nature of vaso-constricting substances, our attention was directed to the amine group.

Dale and Dixon[6] have shown that amines produce a marked rise of blood pressure when injected into the circulation. The following substances were used: aniline, *o*-, *m*- and *p*-aminophenols and *o*- and *p*-hydroxyphenylmethylamines.

Methods. We have restricted ourselves to two methods and consider that when they give positive results that the action may be like adrenalin, negative results prove, however, that the substance tested is not physiologically allied to adrenalin.

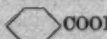
(1) Perfusion of frog's blood-vessels. This was done in the usual way. A frog was chloroformed and then pithed; a cannula was tied in the innominate artery and after the ventricle was cut off the vessels were perfused by a constant head of pressure. The perfusion fluid was a modified Ringer solution containing sodium phosphate, in order to maintain the solution near the neutral point. The drugs were made up in the same solution, the acids in the form of their sodium salts and the amines as the hydrochlorides. The volume of solution flowing through was measured for a convenient interval and then the control solution was replaced by the drug to be tested. After this had perfused for about four minutes and the flow had become regular the flow was again measured; finally Ringer solution was again used to find out if recovery would take place. At the end of the experiment the reaction of the blood-vessels to adrenalin was tested.

(2) Measurement of blood pressure in decapitated cats. The cat was anæsthetised and decapitated by the method described by Sherrington[13], curare was administered and after the blood pressure manometer had been connected to the carotid artery a solution of the

drug was injected into the saphenous vein. A few experiments were also made using anæsthetised cats.

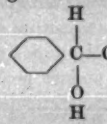
With both these preparations a control of adrenalin was used at the end of the experiment, except in one case where the heart stopped as the result of an injection of catechol.

1. Substances without an Amine Group.

Benzoic acid 

By perfusion M/100. Slight constriction.

By injection 0·3 gm. Rise of 38 mm., Hg. waves appear.

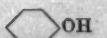
Mandelic acid 

By perfusion M/100. (*a*) Slight dilatation. (*b*) Slight constriction.

M/50. (*a*) Slight constriction. (*b*) Slight dilatation followed by constriction.

By injection 0·3 gm. Rise of 8 mm. Hg.

Monohydroxyphenols.

Phenol 

By perfusion: M/100. Constriction and partial recovery. Adrenalin control positive.

M/50. Dilatation followed by constriction. Adrenalin control positive.

The toxicity of the phenol is shown by the recovery, on re-perfusion of Ringer solution, being only partial.

By injection: 0·01 gm. Rise of 6 mm. Hg., slight muscular tremors and the tracing showed waves after the injection.

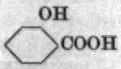
0·03 gm. Rise of 16 mm. Hg. Accompanied by muscular tremors and some irregularity of blood pressure.

0·05 gm. Preparation was in bad condition owing to the previous injection of other substances; fall of 10 mm. Hg.

These results show that phenol in small doses causes a slight rise of blood pressure. The muscular tremors were probably the result of

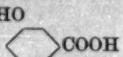
direct action on the muscles as the animals in all these decapitate preparations were curarised. Baglioni[9] on the other hand considers that phenols act on the central nervous system. His comparisons between chemical composition and physiological action are concerned with effects so different from those studied by us that we cannot comment on his results.

Hoppe-Seyler[10] found that small doses cause a rise of blood pressure. Th. Gies[9] obtained a fall of pressure which he said was due to an action on the vaso-motor centre. If the bulb was separated from the spinal cord no effect could be obtained on the blood pressure. As our experiments were performed with headless animals the results support those of Hoppe-Seyler.

o-oxybenzoic acid (*salicylic acid*) 

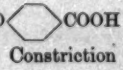
By perfusion M/100. Constriction and recovery.

By injection 0·3 gm. Rise of 4 mm. Hg.

m-oxybenzoic acid 

By perfusion M/100. Constriction and recovery.

By injection 0·3 gm. (*a*) Rise of 54 mm. Hg. Well-marked waves appear. (*b*) Rise of 54 mm. Hg. Amplitude of cardiac pulsations increased.

p-oxybenzoic acid 

By perfusion M/1000. Constriction M/100. Marked constriction and recovery.

By injection 0·3 gm. (*a*) Rise of 6 mm. Hg. Amplitude of cardiac pulsation increased. (*b*) Rise of 16 mm. Hg. Amplitude of cardiac pulsation increased.

Dihydroxyphenols.

Resorcinol

By perfusion M/100. Slight dilatation.

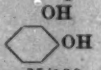
By injection 0·03 gm. Rise of 6 mm. Hg. followed by the appearance of well-marked waves.

0·3 gm. (*a*) Rise of 16 mm. Hg. (*b*) Fall of 20 mm. Hg. Tracing shows a slowing of the heart appearing like heart block and muscular tremors were also seen.

Hydroquinone

By perfusion M/100. Flow reduced but tissues become œdematous and full of mucoid material. No recovery and adrenalin practically inactive.

By injection 0·03 gm. No effect on blood pressure, waves appear.

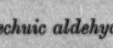
Catechol 

By perfusion M/100. Good constriction.

By injection 0·01 gm. (*a*) Rise of 60 mm. Hg. (*b*) Rise of 14 mm. Hg. (intact animal). (*c*) Rise of 68 mm. Hg. (intact animal). (*d*) Rise of 42 mm. Hg. (intact animal).

0·03 gm. (*a*) Rise of 12 mm. Hg. (*b*) Rise of 10 mm. Hg. (intact animal).

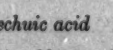
0·3 gm. Sudden permanent stoppage of heart. After the injections the blood became dark in colour, muscular twitching occurs and the heart tends to become irregular and miss beats occasionally.

Protocatechuic aldehyde 

By perfusion M/100. Dilatation. M/50 (*a*) dilatation, (*b*) dilatation.

By injection 0·01 gm. (*a*) Rise of 2 mm. Hg. (*b*) Rise of 6 mm. Hg. (intact animal).

0·3 gm. Rise of 4 mm. Hg.

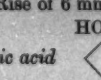
Protocatechuic acid 

By perfusion M/100. Inactive; M/50 (*a*) slight constriction, (*b*) slight constriction.

By injection 0·01 gm. (*a*) No effect. (*b*) No effect (intact animal).

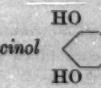
0·02 gm. Rise of 12 mm. Hg. (intact animal).

0·3 gm. Rise of 6 mm. Hg.

β-resorcylic acid 

By perfusion. Slight constriction.

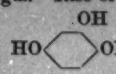
Trihydroxyphenols.

Phluroglucinol 

By perfusion M/100. Dilatation; M/50 some constriction.

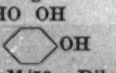
A cloudy protein-like precipitate appeared in the fluid after perfusion. The tissues became œdematous.

By injection 0·03 gm. Rise of 10 mm. Hg. blood becomes dark.

Oxyhydroquinone 

By perfusion M/100. Good constriction. Œdema, bad recovery and adrenalin control poor.

By injection 0·3 gm. Fall of 16 mm. Hg., blood becomes dark.

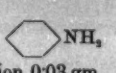
Pyrogallol 

By perfusion M/50. Dilatation and œdema, good adrenalin control.

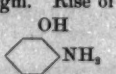
By injection 0·03 gm. Fall of 4 mm. Hg.

0·15 gm. Fall of pressure and condition like heart block.

2. Substances containing an Amine Group.

Anilene 

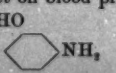
By injection 0·03 gm. Rise of 6 mm. Hg.

o-aminophenol 

By injection 0·01 gm. Rise of 8 mm. Hg.

0·03 gm. (*a*) Rise of 2 mm. Hg. (*b*) Rise 8 mm. Hg.

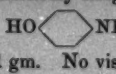
0·2 gm. No effect on blood pressure.

m-aminophenol 

By injection 0·01 gm. No visible effect.

0·03 gm. (*a*) No visible effect. (*b*) Rise of 14 mm. Hg.

0·2 gm. Heart markedly irregular and beats missed.

p-aminophenol 

By injection 0·01 gm. No visible effect.

0·03 gm. (*a*) Rise of 6 mm. Hg. (*b*) Rise of 16 mm. Hg. (Fig. 1).

0·2 gm. Heart irregular and misses beats.

The aminophenols show a tendency to produce waves on the blood pressure tracing and in one experiment when the three had been injected

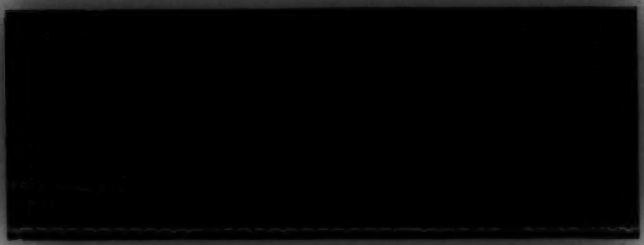
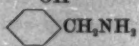


Fig. 1. Effect of injection of 0·05 gm. *p*-aminophenol. Time markings 1″.

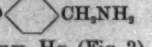
Fig. 2. After-effect of injection of the three aminophenols into the same animal. The drum was stopped between the rises of pressure and started again just as the pressure commenced to rise. Probably due to discharges from spinal vaso-motor centres. Time markings 1″.

Fig. 3. Effect of injection of 0·01 gm. *p*-hydroxyphenylmethylamine. Time markings 1″.

into the same preparation rises of about 16mm. Hg. occurred at intervals of 2, 4, 4, 4, and six minutes (Fig. 2).

o-hydroxyphenylmethylamine 

By injection 0·01 gm. Rise of 60 mm. Hg.

p-hydroxyphenylmethylamine 

By injection 0·01 gm. Rise of 68 mm. Hg. (Fig. 3).

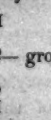
Summary of Results.

In the absence of the amine group there are only three of the substances used that had any appreciable effect on the blood pressure, these substances were benzoic acid, *m*-oxybenzoic acid and catechol and in the last the two hydroxyls are in the same relative position to each other as they are in adrenalin.

The phenolic bodies are on the whole toxic in action but they exhibit very well the influence of the relative position of the groups on their behaviour in the body. The differences between the three oxybenzoic acids is noticeable.

The three dihydroxyphenols show the same relative order of toxicity as that given by Fränkel[9] (catechol, hydroquinone, resorcinol as 10 : 5 : 1) and it is remarkable to see how the addition of an aldehyde or acid group to the most toxic of these abolishes the toxic action.

The trihydroxyphenols are less toxic than the dihydroxy compounds, With many of the substances used the blood became dark in colour, muscular tremors occurred and waves appeared on the blood pressure tracing. These waves were probably due to stimulation of subsidiary vaso-motor centres in the spinal cord. In one experiment these waves seemed to take the form of explosive discharges at more or less regular intervals (Fig. 3). Irregularity of the heart and a condition resembling heart block were seen in many of the tracings.

The presence of the  group in mandelic acid showed no trace of the marked effect produced by reduction of the ketonic substances of allied composition to adrenalin.

Amongst the substances containing an amine group anilene and the aminophenols caused very little rise of pressure and the latter substances were less toxic than the anilene. The placing of a CH_2 group between the benzene ring and the NH_2 group caused a marked effect on the activity of the resulting compound.

After the experimental portion of this paper was completed Barger and Dale[3] published their results on the physiological action of amines. Their conclusions agree with ours in ascribing the main factor in the "sympathomimetic" action to the presence of an amine group separated from the benzene ring by a carbon chain.

They find that when two phenolic hydroxyls are present in the 3 : 4 position the presence of an asymmetric alcohol group makes the action stronger than the corresponding ketone, but that when the catechol nucleus is not present reduction of the ketone does not increase the activity of the resulting compound.

They also point out that the rise of pressure with catechol is not "sympathomimetic" and thus it appears probable that the rises of pressure with benzoic acid and *m*-oxybenzoic acid are also not "sympathomimetic."

They state that a phenolic hydroxyl in the ortho position does not increase the activity but we found that *o*-hydroxyphenylmethylamine is as active as the para compound.

The following comparison is of interest. Barger and Dale found that anilene and phenylmethylamine were practically without effect whilst β-phenylethylamine was markedly active. In our experiments anilene and the aminophenols were ineffective and the two hydroxyphenylmethylamines tried caused a large rise of blood pressure.

Both these results point to the great influence of an added $-CH_2-$ group but if phenylmethylamine is compared with the hydroxyphenylmethylamines the facts could be explained as due to the addition of a phenolic hydroxyl. It seems that these relationships demonstrate the interaction of one group on another and thus by the addition of an hydroxyl the activity is increased so as to correspond to a higher member of the series containing one more $-CH_2-$ group. Barger and Dale point out similar interactions between the secondary alcohol compounds and the simple carbon chain depending on the presence or absence of phenolic hydroxyls (p. 29).

Our results together with those of the other workers mentioned seem to warrant the following deductions about the action of adrenalin :

1. The activity of adrenalin is largely dependent on the presence

of an amine (or substituted amine) group separated from the benzene nucleus by some other group.

2. Two hydroxyls attached to the benzene ring in the ortho (3 : 4) position increase the action and when these are present the placing of a secondary alcohol group between the benzene ring and the amine-containing group intensifies the activity.

3. In the latter case it is the lævo-rotatory compound that is mainly responsible for the "sympathomimetic" action.

APPENDIX.

Preparation of p-hydroxyphenylmethylamine.

5 gm. *p*-hydroxybenzaldehyde were treated with the calculated amount of sodium bisulphite. The addition-product was suspended in water and treated with 5 gm. potassium cyanide and 2·5 gm. HCl, when the nitrile separated out as a brown oil. The crude nitrile was reduced with zinc and HCl. The reduction-product solidified on standing (2 days) and crystallised from water in small cubes. M.P. 234—236° C.

Analysis:

C_7H_7ON requires C = 69·42, H = 5·7.
Found C = 70·23, 70·17, N = 11·57.
H = 6·21, 6·23.
N = 11·71, 11·62.

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THE PHYSIOLOGICAL ACTION OF β-IMINAZOLYLETHYLAMINE.

By H. H. DALE and P. P. LAIDLAW.

(*From the Wellcome Physiological Research Laboratories.*)

β-IMINAZOLYLETHYLAMINE is the amine which is produced when carbon dioxide is split off from histidine. It was first prepared synthetically by Windaus and Vogt[1]. Recently Ackermann[2] obtained a large yield of the base by submitting histidine to the action of putrefactive organisms. It has been shown that several of the amines thus related to amino-acids possess marked physiological activity. The activity of β-iminazolylethylamine was discovered in the course of the investigation of ergot and its extracts by G. Barger and one of us[3], who attributed this structure to a base which they obtained, and which in minute doses produced tonic contraction of the uterus. The synthetic substance, and the base produced by splitting off carbon dioxide from histidine by bacterial action or by chemical means, were found to have an identical action. Meanwhile Kutscher[4] had simultaneously and independently described the isolation from ergot of a base having this action and presumably identical with that obtained by Barger and Dale. By its chemical properties this first ergot base of Kutscher was not distinguishable from β-iminazolylethylamine; but certain apparent differences in the physiological action of the two bases, observed by Ackermann and Kutscher[5], led them to the conclusion that the ergot base, though closely related to β-iminazolylethylamine, is not identical with it. The alleged difference in action, on the existence and cause of which our experiments throw light, was as follows: the

[1] *Ber. d. deutsch. chem. Gesell.* XL. p. 3691. 1907.

[2] *Zeit. f. physiol. Chem.* LXV. p. 504. 1910.

[3] *Proc. Chem. Soc.* XXVI. p. 128. 1910. This *Journal*, XL. Proc. Phys. Soc. p. XXXVIII. 1910.

[4] *Zentralbl. f. Physiol.* XXIV. p. 163. 1910.

[5] *Zeit. f. Biol.* LIV. p. 387. 1910.

ergot base, injected intravenously into a rabbit in a dose of a few milligrammes, caused stoppage of respiration and fall of blood-pressure terminating in fatal heart failure; whereas iminazolylethylamine, under the same conditions, caused always a rise of blood-pressure, an injection of even 200 mgms. not being fatal.

In the present paper we are not directly concerned with this question of the identity of the ergot base, and shall refer to it only in so far as our own experiments have a bearing on the alleged difference. We may note, however, in passing, that the case for identity of the two substances receives some slight support from the more recent isolation from ergot extract, by Engeland and Kutscher[1], of a "second active base," having an action in some points similar to the first. This second base they have identified as the analogous amine from arginine, previously prepared by Kossel[2], who named it "Agmatine." All the experiments here described were made with β-iminazolylethylamine prepared from histidine by a chemical process, which will be described elsewhere by Mr Ewins, to whom we are indebted for our supply. A few confirmatory experiments were made with the synthetic base, which Mr Ewins also prepared by Windaus and Vogt's method, and with the base prepared from histidine by putrefaction, for which we are indebted to the kindness of Dr Ackermann.

No conveniently short name being yet available we shall refer to the base in this paper as β-I. The hydrochloride was used in all our physiological experiments, and weighed without allowance for the difference in molecular weight. "One mgm. β-I." is, therefore, to be taken to mean one mgm. of the hydrochloride.

Action on the intact animal.

The Frog The frog is but slightly affected by β-I. Injections of one to ten mgms. into the dorsal lymph-sac caused gaping movements of the lower jaw, succeeded by depression of the central nervous system for periods increasing with the dose, the effect lasting for about 30 mins. after ten mgms. During this period the animal sprawls in a relaxed attitude and the legs are not withdrawn when passively extended. Recovery is rapid and complete and the effect is in no way peculiar to or characteristic of β-I.

[1] *Zentralbl. f. Physiol.* XXIV. pp. 479 and 589. 1910.
[2] *Zeitsch. f. physiol. Chem.* LXVI. p. 257. 1910.

Mammalia. In rodents the effects are very different in the case of intravenous and of subcutaneous injection. In a rabbit of medium size an injection of two mgms. intravenously (ear-vein) caused marked prostration, the respiration becoming irregular and laboured and the heart-beat intermittent and feeble. These effects passed off gradually and recovery took place if no further injection were given. A second dose of two mgms., injected intravenously before the effects of the first had wholly subsided, caused a renewal of the symptoms in an accentuated form, death ensuing in a few seconds. Spasmodic inspiratory efforts continued for some time after cessation of the heart-beat. *Post-mortem* the right side of the heart was found greatly engorged: the lungs were slightly distended, but not otherwise abnormal. Death was apparently due, therefore, to right-sided heart-failure, associated with, but apparently not wholly dependent on, respiratory disturbance. Larger doses of five to ten mgms., injected intravenously, caused rapid death with similar symptoms, convulsive and obstructed respiration being a prominent feature of the effect.

In large guinea-pigs, weighing 800—1000 grams, injection of 0·5 mgm. into the external saphena vein caused death in a few minutes. The immediate effect was a marked respiratory impediment, resulting in violent but largely ineffective inspiratory efforts, during which the lower ribs were drawn in. After a time the respiratory convulsions ceased, and the animal lay comatose, though the heart continued beating for some time longer. *Post-mortem* the lungs were found permanently distended. If the fatal amount were given more slowly, as in two doses of 0·25 mgm. after the second of which death ensued rapidly, the final condition of pulmonary distension was extreme. Death was clearly due to asphyxia, evidently resulting from progressive obstruction to the respiration, sufficient in its early stages to prevent the exit of the air sucked into the lungs by the violent inspiratory spasms, and later becoming complete. The larger the initial dose, and, therefore, the earlier the obstruction became complete, the less pronounced the distension of the lungs. Such an effect could only be due to constriction of the bronchioles by spasm of their muscular coats, though the effect would be aided by increased bronchial secretion. Preliminary injection of atropine, though it did not abolish the action, had decided protective value. After five mgms. of atropine a dose of one mgm. of β-I. intravenously had the normal fatal effect: but another guinea-pig, which received a preliminary injection of five mgms. of atropine, recovered from subsequent intravenous injections of 0·5 mgm.,

0·25 mgm., and again 0·5 mgm. of β-I., given in fairly rapid succession; whereas one dose of 0·5 mgm. was, in our experience, invariably fatal when given intravenously to a guinea-pig untreated with atropine. Whether atropine actually weakens the bronchial spasm, or merely modifies the effect by preventing secretion, must remain uncertain. We were unable to remove the obstruction, when once developed, by a subsequent injection of atropine.

It may be noted, at this point, that the symptoms and *post-mortem* condition in the guinea-pig correspond in a suggestive manner with those described by several observers[1] as the effects of poisoning in that animal by Witte's peptone, or by serum or other protein in the sensitised guinea-pig ("anaphylactic shock"). Also that the dose of iminazolylethylamine which proved fatal in our experiments to the unanæsthetised rabbit, when given intravenously, is practically identical with that given by Ackermann and Kutscher[2] as the fatal dose of Kutscher's first ergot base for the rabbit. To both these points we shall return later.

When the injection is made subcutaneously much larger doses are easily tolerated, both by the rabbit and the guinea-pig. 25 mgms. thus administered to a rabbit caused a gradual increase in rate of both heart-beat and respiration, the effect first becoming marked about 15 minutes after the injection. Defæcation, with semi-fluid fæces, and micturition occurred, and during the hour succeeding the onset of the symptoms the animal showed signs of prostration, with moderately deep narcosis. The attitude was sprawling, the head sunk on the table, the ears pale and cold. Recovery then set in, and in a few hours the animal was apparently normal.

In the cat the discrepancy between the effects of intravenous and subcutaneous injections was not so marked. Intravenous injections (long saphena vein) of two, four, eight and ten mgms. caused immediate vomiting and purging, profuse salivation, and laboured respiration, with a subsequent period of collapse and light narcosis, increasing with the dose. During this narcotic stage the pupils were markedly constricted. The cat which received ten mgms. intravenously, and which at the end of an hour had partially recovered, was then given a second intravenous injection of 20 mgms. This caused renewed vomiting and collapse, but the symptoms were rather less marked than those produced by the

[1] Cf. Biedl and Kraus. *Zentralbl. f. Physiol.* XXIV. p. 258. 1910; and Auer and Lewis, *Journ. exper. Med.* XII. p. 151. 1910.

[2] *loc. cit.*

first injection of ten mgms. The animal, which had thus received 30 mgms. in all intravenously, recovered completely during the night. The effects of subcutaneous injections in the cat were similar, though naturally somewhat slower in onset. 50 mgms. injected into a cat subcutaneously produced vomiting in six minutes, followed by purgation, and subsequently collapse and mild narcosis very similar to the condition following smaller intravenous injections. The constriction of the pupils in the narcotic stage was strongly marked even in the shade, but dilatation could be produced by rousing the animal. Complete recovery followed.

Another cat, a female in late pregnancy, received a subcutaneous injection of 50 mgms. of β-I., the general symptoms which resulted being very similar to those above described. Periodical strong contractions of the uterus, alternating with periods of relaxation, could be observed by palpation of the abdomen. During the night one of the two fœtuses was born dead. On the following day a second injection of 100 mgms. was given, which produced a repetition of the symptoms. The second fœtus was born, also dead, 5½ hours after this second injection. The mother recovered completely and was quite normal on the following day. It seems probable that the powerful incoordinate contractions of the uterus, though they had no expulsive value, caused separation of the placentæ and asphyxiation of the fœtuses, which were expelled by the normal action of the uterus when the direct effect of the drug on that organ had passed off.

The vascular system.

The effect of iminazolylethylamine on the arterial blood-pressure is complex and not easily interpreted. It not only varies in different species, but shows very wide variations in individuals of the same species, especially in rabbits. These variations appear altogether out of proportion to the small differences of experimental conditions, and it is probable that individual differences of sensitiveness are also concerned.

The Cat and Dog. Dealing first with the cat and dog, in which the effects are relatively constant, we find that in these animals, anæsthetised with morphia and A.C.E. mixture, with paraldehyde or urethane, or by pithing the brain, the effect of injecting a small dose of β-I. intravenously is almost always a considerable fall of systemic arterial pressure. This was also described by Ackermann and

Kutscher[1]. If the dose is small and the arterial pressure low, as in a pithed animal towards the end of a long experiment, the fall may be very slight and succeeded by a small rise of pressure, which is then the main effect (cf. Fig. 14). The normal and characteristic effect in these species, however, is a fall of pressure, which can sometimes be observed to occur in two stages, a preliminary fall, lasting about ten seconds, being succeeded by a more marked secondary fall, the duration of which varies with the dose (cf. Fig. 1). With doses of 1 mgm. or more the secondary fall is much prolonged, the pressure only returning very gradually to the original level.

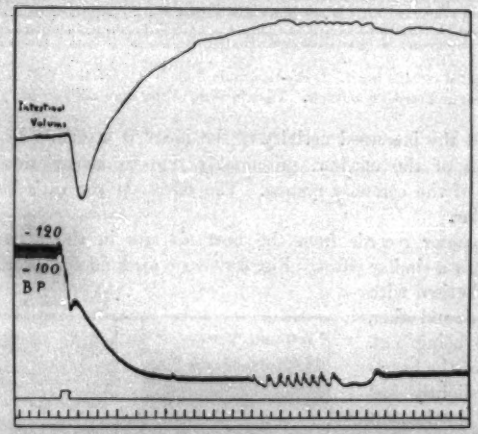

Fig. 1. Cat. Decerebrate. Effect of 1 mgm. β-I. on intestinal volume and blood-pressure. In this tracing, and following ones unless specially mentioned, the time marker intervals of 10″.

The significance of the different phases of this effect was investigated by examining separately the action on (*a*) the heart, (*b*) the pulmonary vessels, (*c*) the systemic vessels.

(*a*) The fall of systemic arterial pressure is not due to direct depression of the heart's activity. The action of iminazolylethylamine on the isolated, artificially perfused heart of the rabbit or cat is, indeed, to produce increase in both the rate and the force of the beat.

[1] *loc. cit.*

Fig. 2 shows the effect of injecting 0·25 mgm. of the hydrochloride into the cannula from which a rabbit's heart is perfused with warm oxygenated Ringer's solution, the substance being dissolved for injection in the same solution. The drop-record of the coronary flow, obtained by collecting the irregular spurts and drippings from the heart into a regular series of large drops, as previously described by one of us,

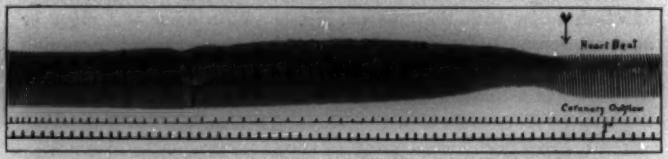

Fig. 2. Isolated rabbit's heart. Locke-Langendorff method. Effect of 0·25 mgm. β-I. on heart beat and coronary outflow. Time in secs. Read from right to left.

shows that the increased activity of the heart is attended by distinct retardation of the outflow, presumably due to constriction of the arterioles of the coronary system. The effect on the cat's heart was very similar.

Cardiometer records from the heart *in situ* in the anæsthetised animal show a similar effect. Fig. 3 shows a cardiometer record from a cat anæsthetised with paraldehyde and ether, the vagi being cut. The rate of the heart-beat is slightly less after the injection, but the output per beat is more than proportionately increased, so that the output per unit time is greater, though the blood-pressure falls. The slowing of the beat in this case is probably explicable as a weak pilocarpine-like action, which β-I. also shows in other directions. As we have seen, this effect is absent in the case of the isolated organ, the

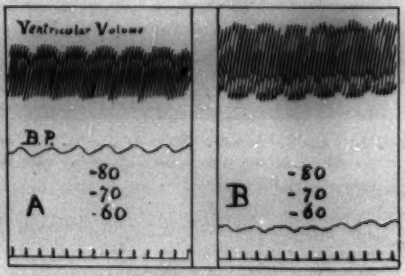

Fig. 3. Cat. Paraldehyde and ether. Time in secs. *A* normal: *B* maximal effect of 0·25 mgm. β-I. cardiac output and blood-pressure.

directly stimulating effect of the base on the heart-muscle having free play. In any case, since the beat of the heart, even when slowed, is augmented sufficiently to increase the rate of output, the fall of blood-pressure must be due to something other than an action of the drug on either the muscle or the peripheral nervous mechanism of the heart. When large doses are given the large fall of pressure causes secondary weakening of the heart-beat, presumably due to anæmia of the muscle, resulting from the association of coronary constriction with falling arterial tension. This secondary weakening then doubtless becomes a factor tending to accentuate and prolong the depressor effect of such doses.

(*b*) *The pulmonary vessels.* The effect on these was examined by recording the pulmonary blood-pressure, and by artificial perfusion of the surviving lung. The pressure was recorded from a branch of the pulmonary artery of a cat anæsthetised with paraldehyde and ether,

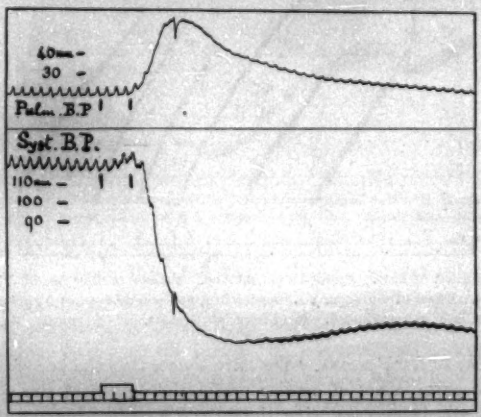

Fig. 4. Cat. Paraldehyde and ether. Time in 2″. Effect of 0·5 mgm. β-I. on pulmonary and systemic blood-pressures.

a mercury manometer being used. The method of operation was that described by Bradford and Dean[1]. The carotid blood-pressure was recorded simultaneously by a second manometer. Fig. 4 shows the effect of injecting 0·5 mgm. β-I. intravenously in this experiment. It

[1] This *Journal*, XVI. p. 34. 1894.

will be seen that a rise of pulmonary pressure, amounting to about 40 mm. at its maximum, follows closely on the injection, its commencement preceding that of the systemic fall by about two seconds. Since the action on the heart is but slight, this large rise of pulmonary pressure can only be attributed to constriction of the pulmonary arterioles. The perfusion with recorded rate of outflow was performed by Brodie and Dixon's[1] method, the lung used being taken from a cat killed by pithing. The perfusion fluid was the animal's own defibrinated blood diluted with an equal volume of Ringer's solution. Injection of 0·25—0·5 mgm. β-I. into the inflowing perfusion fluid, at a point near the arterial cannula, regularly produced a pronounced diminution in the rate of outflow from the venous side (Fig. 5). This, again, can only be attributed to constriction of the pulmonary arterioles.

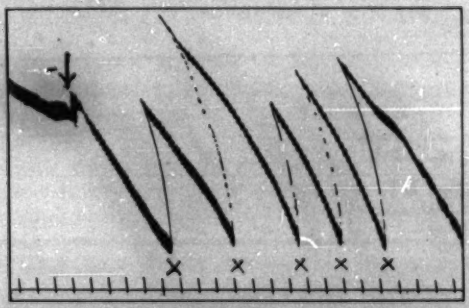

Fig. 5. Perfusion of lung. Brodie-Dixon method. Venous outflow recorded by bellows recorder. Effect of 0·5 mgm. β-I. injected into perfusion cannula: at points marked × the bellows was readjusted. Read from left to right.

(c) *The systemic vessels.* We have shown that the fall of systemic arterial pressure is not of cardiac origin, the output of the ventricles being actually increased during the fall. The effect of the pulmonary constriction, therefore, in diminishing the output of the left ventricle, when its effect is perceptible at all, must be limited to the initial stage of the systemic fall. This is clear also from the time relations of the two effects, the pulmonary rise of blood-pressure having already passed its maximum when the systemic fall is but beginning. In one experiment the drug was injected into the central end of one carotid artery, the other

[1] This *Journal*, XXX. p. 476. 1904.

being used for recording the blood-pressure; and it was found that, when the injection was thus made into the main arterial stream, the fall of pressure began earlier than when the injection was made as usual into a systemic vein. In this case, then, the effect of the pulmonary constriction on the systemic pressure was negligible, and the fall must be regarded as wholly due to dilatation of the systemic arterioles. The time relations of the effects suggest, however, that, when the fall of pressure occurs in two phases, the preliminary phase is due to pulmonary constriction reducing the output from the left side of the heart. This is confirmed by plethysmographic observations. In Fig. 1 the first phase of the fall is seen to be associated with a distinct

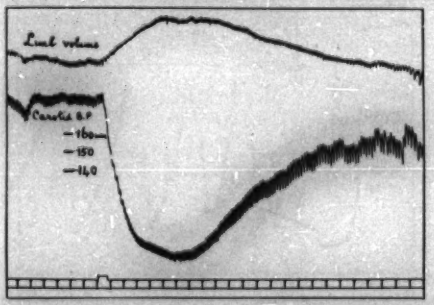

Fig. 6. Cat. Ether. Effect of 0·5 mgm. β-I. on limb-volume and systemic blood-pressure.

decrease in the volume of a loop of intestine enclosed in an air plethysmograph: as the second and main phase of the fall develops the intestinal volume undergoes a large expansion. When the first phase of the fall is not present the increase of intestinal volume either begins with the fall, or is preceded only by such a small preliminary diminution as is explicable by the less ready filling of the vessels of the loop, which is raised in the plethysmograph somewhat above the general level of the abdominal viscera. (Cf. Fig. 7.) The main effect, in any case, is an increase of intestinal volume corresponding to the fall of pressure, both returning to the normal *pari passu*.

Vaso-dilatation, corresponding to the fall of arterial pressure, also occurs in the limbs. The plethysmographic demonstration of this is not quite so simple, since the circulation in the limbs of an anæsthetised animal is apt to become sluggish, and the great fall of arterial pressure,

due mainly to vaso-dilatation in the splanchnic area, may mask the effect of the local vaso-dilatation in the limb. The normal effect, however, is that illustrated in Fig. 6, which is quite decisive.

The case of the kidney is different. Injection of β-I. regularly causes a large decrease of volume in this organ (Fig. 7): this is the case

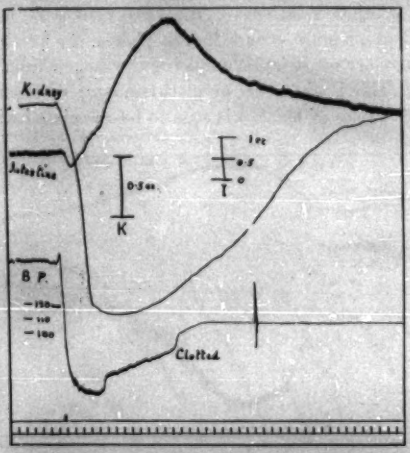

Fig. 7. Cat. Paraldehyde and ether. Effect of 0·5 mgm. β-I. on kidney volume, intestinal volume and blood-pressure. *K* and *I* are calibrations of recorders used to estimate changes in kidney volume and intestinal volume respectively.

even when the limbs show a good expansion. This decrease in the volume of the kidney appears too great to be wholly a passive effect of the fall of arterial pressure, and suggests an active constriction of the arterioles of the kidney: but this cannot be regarded as demonstrated.

The plethysmographic results thus show clearly that the fall of arterial pressure is mainly due to a general vaso-dilatation, in which the arterioles of the kidney do not participate: but the mechanism of this dilatation is not perfectly clear. When the vessels of the small intestine of a cat or dog, or of the hind limbs of a cat, were artificially perfused by Brodie and Dixon's method, the rate of venous outflow being recorded, an entirely different result was obtained. The perfusion-fluid used was the animal's own blood, defibrinated by whipping and diluted with an equal volume of Locke-Ringer solution. Injection of β-I. into the

arterial side of the system, close to the arterial cannula, in doses varying of 0·1—0·5 mgm., caused regularly a pronounced retardation of the venous outflow. (Fig. 8.) The hydrochloride was dissolved in a small quantity of the perfusion fluid, and injected at the temperature of that in the arterial tube, so that viscosity and temperature effects were excluded. The muscular coats of the intestinal wall appear to be rather more sensitive to β-I. when isolated from the body; but this difference would be quite inadequate to explain the actual reversal of the effect, and in any case could not account for the similar discrepancy between the effects during life and during artificial perfusion seen in the case of the limb-vessels. There is, therefore, no escape from the conclusion that β-I. has a vaso-dilator effect when injected into the animal, a vaso-constrictor effect when perfused through isolated organs.

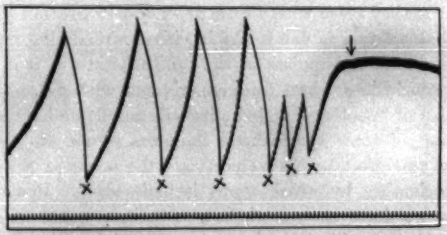

Fig. 8. Perfusion of small intestine. As in Fig. 5 at ↓ 0·1 mgm. β-I. Read from right to left.

In other words the systemic arterioles respond to the drug, like most other plain muscle, by increase of tonus when isolated from the body. Their dilatation in the body must, therefore, be attributed to some intermediate mechanism which does not survive excision and artificial perfusion.

Concerning this vaso-dilator mechanism we know definitely only that it is localised at the periphery. After section of the splanchnics, or injection of nicotine in doses sufficient to block impulses through autonomic ganglia (30 mgms. for a cat), vaso-dilatation was still produced by β-I. in the intestinal vessels. In a cat under ether the right stellate ganglion was removed by Anderson's method. The animal was then turned on its back, and records taken of the carotid blood-pressure and volume of the right fore-limb. Injection of 0·5 mgm. β-I. caused the usual fall of blood-pressure and quite normal expansion of the limb.

We have previously referred to the analogy between the effect of β-I. and that of Witte's peptone, as described by various observers. Thompson[1] attributed the fall of pressure caused by "peptone" to loss of "vasomobility," shown by loss of the normal constrictor response to stimulation of the splanchnic nerves. It is of interest to note, incidentally, that he found the kidney vessels immune to this vaso-dilator action of peptone, as we found them to that of β-I. More recently Popielski[2] has attributed the vaso-dilator action of peptone, and of other preparations containing his hypothetical "vaso-dilatin," to blocking of tonic sympathetic impulses by paralysis of peripheral nervous structures: since he found that adrenine caused a rise of pressure after "vaso-dilatin" he regarded the former as acting more peripherally. On the other hand, Biedl and Kraus[3] state the vaso-dilatation caused by "peptone" does neutralise the pressor effect of adrenine; but they agree with Thompson and Popielski in regarding the vaso-dilator action as due to the blocking or rendering ineffective of tonic vaso-constrictor impulses in the sympathetic. If it is legitimate to transfer conclusions drawn from experiments with β-I. to the closely similar action of "peptone" and similar substances, we have shown that this blocking of tonic sympathetic impulses is not an adequate explanation of the effect, unless we postulate the presence of a peripheral nervous mechanism by which tonus is maintained. Removal of the ganglia, as we have shown, whether actual or functional, does not prevent further vaso-dilatation in response to β-I. Whether the dilatation is due to action on peripheral nerve-endings can only be settled by degeneration experiments which we hope shortly to undertake. Experiments by Hamburger[4] appear to show that post-ganglionic degeneration of the sympathetic nerve-supply destroys the vaso-dilator action of "peptone." However that may be, the facts available appear to indicate that the depression of the adrenine effect, which is also seen with β-I., is due to the antagonism of an active dilatation, not to the paralysis of a receptive mechanism common to the two.

The Rabbit. The effect of β-I. on the blood-pressure of the rabbit is greatly modified by the respiratory effects, which, as we have noticed in describing the action on the intact animal, are much more marked in

[1] This *Journal*, xx. p. 455. 1896: xxiv. p. 374. 1899: and xxv. p. 1. 1899.

[2] *Arch. f. exp. Path. u. Pharm.* (*Schmiedeberg Festschr.*) p. 437. 1908. *Pflüger's Arch.* cxxvi. p. 483. 1908.

[3] *Wien. klin. Wochenschr.* xxii. 1909.

[4] *Amer. Journ. of Phys.* xi. 282. 1904.

rodents than in carnivora. For this reason the condition of anæsthesia, and the length of time during which the animal has already been under the anæsthetic when the injection is given, have a considerable influence on the result. It was pointed out by Brodie and Dixon[1] that the response of the bronchial muscles is depressed by various anæsthetics, especially by urethane, which can even obscure the bronchio-constrictor effect of muscarine. We have seen that bronchial spasm is a typical feature of the action of β-I. on the unanæsthetised rabbit or guinea-pig. We have also seen that, under these conditions, β-I. is as fatal to the rabbit as Ackermann and Kutscher found the ergot-base. It seemed

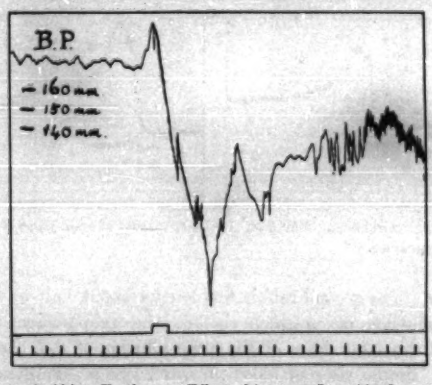

Fig. 9. Rabbit. Urethane. Effect of 1 mgr. β-I. on blood-pressure.

possible, therefore, that the relatively harmless effect of β-I. in their experiments might be connected with their use of urethane as an anæsthetic. We therefore made observations on the blood-pressure of rabbits anæsthetised with urethane for varying periods, and a comparison of the results of the two following experiments is instructive. Each of two rabbits was anæsthetised by an injection of urethane solution, 1·5 grms. per kilo. being given in each case. In the first rabbit, as soon as complete anæsthesia was produced, the operation of inserting the necessary cannulæ was rapidly completed. The record was then started and an injection of 1 mgm. β-I. given intravenously. Fig. 9 shows the effect on the carotid blood-pressure. It will be seen

[1] This *Journal*, XXIX. p. 97. 1903.

that the pressure begins to rise, but the rise soon gives way to a rapid fall. During this fall vigorous but largely ineffective inspiratory efforts occurred, the resistance to air entry being obviously very great. Heart-failure seemed imminent, but recovery soon set in and the blood-pressure again became steady at a rather lower level than that obtaining at the beginning of the experiment. A second injection of 2 mgms. was then given: violent and ineffective respiratory efforts immediately reappeared, during which the blood-pressure fell rapidly and permanently to zero, the force of the artificial respiration pump, which was applied, being insufficient to produce an effective expansion

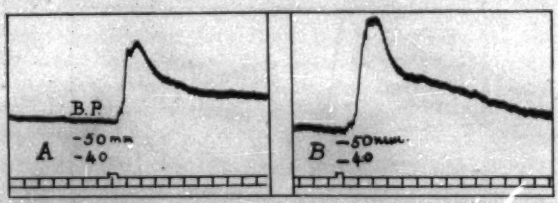

Fig. 10. Rabbit. Urethane. Effect of, *A* 0·1 c.c. extract of ergot; and *B* 1 mgr. β-I. on the blood-pressure.

of the lungs. The second rabbit was left for about half an hour under the full influence of urethane before the preparation was begun. Injection of the same doses of the same solution of β-I. caused, in this experiment, simple rises of blood-pressure. Some temporary increase of resistance to air-entry, with deepened respiration, was manifest, but not sufficient seriously to interfere with the circulation. Larger doses in this rabbit simply caused more prolonged rises of blood-pressure. Fig. 10 shows the effect on the blood-pressure of a rabbit, after prolonged urethane anæsthesia of β-I. and of an ergot extract having a very powerful stimulant action on the isolated uterus. It seems clear that the uncomplicated effect of β-I. on the heart and vessels of the rabbit is to cause a rise of systemic pressure. This can be shown by the plethysmograph to be due mostly to arterial constriction, in which, in this animal, the intestinal vessels share. (Fig. 11.) Unless, however, the effect on the bronchioles be depressed, as by prolonged urethane anæsthesia, this effect is overshadowed by the secondary effects. The sudden and even fatal fall of pressure, produced under such conditions by small doses of the drug, may probably be attributed to the combined

effects of the sudden reduction of intrathoracic pressure by obstructed inspiratory spasms and of constriction of the pulmonary arterioles, the two together causing acute distension of the right side of the heart, terminating in heart-failure.

We have not done sufficient experiments of this kind to be able to state positively that the difference is entirely due to difference in the duration of the anæsthesia prior to the injection. Not improbably the age of the animal, or idiosyncrasy for the drug may be an important factor. However that may be, our experiments show that it is possible with β-I. to obtain in different rabbits results differing from one

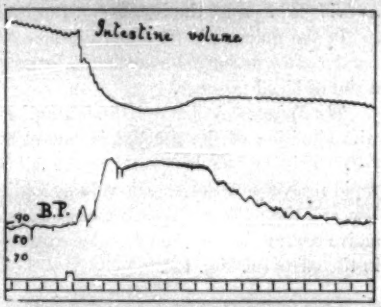

Fig. 11. Rabbit. Urethane. Effect of 1 mgr. β-I. on blood-pressure and intestinal volume.

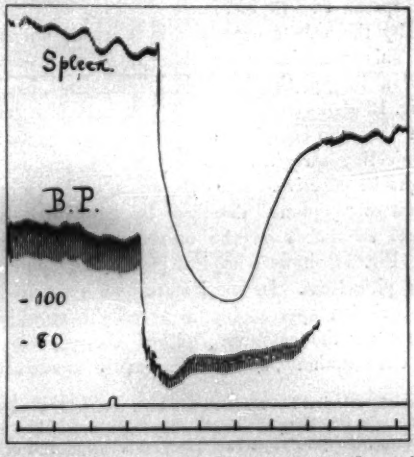

Fig. 12. Cat. Decerebrate. Time in 30". Effect of 0·5 mgr. β-I. on spleen volume and blood-pressure.

another as widely, and in the same manner, as the results obtained by Ackermann and Kutscher with the ergot base on the one hand, and β-I. on the other. Their results cannot, therefore, be properly used as evidence against the identity of the bases[1].

In the guinea-pig we found likewise that, when the bronchioles are paralysed by prolonged anæsthesia, intravenous injection of β-I. causes a rise of blood-pressure.

The Spleen. A marked diminution of volume of the cat's spleen, with abolition of the rhythm, is caused by an intravenous injection of β-I. This may be due partly to the fall of arterial pressure: but the rapid recovery of the spleen volume while the blood-pressure remains low, and the puckered appearance of the surface of the organ, show that active contraction of the muscular capsule must be the principal factor in the effect (cf. Fig. 12).

The respiratory system.

The most obvious of the respiratory effects has already been mentioned, namely, the acute obstruction of air-entry seen in rodents, and especially in the guinea-pig. This must be attributed primarily to bronchial spasm, though increased secretion of bronchial mucus would intensify the effect, especially in a small animal such as a guinea-pig. This bronchial constriction can be mechanically recorded by means of a lung-plethysmograph, as described by Brodie and Dixon[2]. Fig. 13 shows such a record from a pithed guinea-pig. It will be seen that the complete obstruction, once produced, was not relaxed by injecting atropine. Possibly with a smaller dose of β-I. atropine might be effective. If so the effect would be in all respects similar to that of "peptone" described by Biedl and Kraus[3].

In the cat the effect on the bronchioles is usually much less pronounced. Fig. 14 shows a typical plethysmographic record from the lung of a pithed cat. In one instance we observed in the cat a bronchial constriction approaching in severity that which occurs in rodents. Since all the cats were pithed the variation cannot be attributed to the anæsthetic, and the abnormally intense action must be

[1] Recently the physiological identification has been completed by experiments which Barger and Dale are publishing elsewhere. It was found that in a rabbit, after an hour under urethane, the pure ergot base produced a simple rise of blood-pressure, indistinguishable from that produced by β-I.

[2] *loc. cit.* [3] *loc. cit.*

attributed to idiosyncrasy. We could find no evidence that atropine, previously injected, in doses up to 5 mgms., prevented the constrictor effect of β-I. on the cat's bronchioles, or accelerated recovery from the effect when injected subsequently to its production.

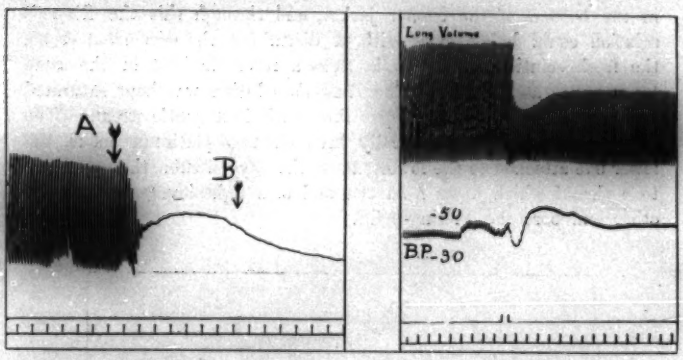

Fig. 13. Guinea-pig. Pithed. Effect of 0·5 mgr. β-I. on lung volume at *A*. At *B* 5 mgrs. of atropine.

Fig. 14. Cat. Pithed. Effect of 0·5 mgr. β-I. on lung volume and blood-pressure.

The local effect on the bronchioles produces such disturbance of the respiratory rhythm that it is not possible to state definitely whether β-I. has a direct effect on the respiratory centre. Large hypodermic injections, causing no serious impediment to the air-entry, are, indeed, followed by slowing and deepening of the respiration. This, however, may be secondary to the changes of blood-pressure.

The uterus.

The intense stimulating action of β-iminazolylethylamine on the muscle of the uterus is the characteristic which first drew attention to its physiological activity. The use of the isolated uterus, preferably of a non-pregnant cat, as a test-object for ergot preparations, was first suggested by Kehrer[1]. In our own experiments we have found the uterus of the virgin guinea-pig the most sensitive and convenient form of plain muscle for exhibiting the action of β-I. A horn of the uterus

[1] *Arch. f. exp. Path. u. Pharmakol.* LVIII. p. 366. 1908.

was suspended in a glass vessel holding 250 c.c. of Ringer's solution, the temperature of which was maintained steadily at 38°—39° C. by means of an outer copper vessel filled with water, the temperature of which was regulated by the method described by Locke. A tube leading from the lower end of the Ringer bath passed through a cork in the bottom of the copper jacket, and through this the Ringer's solution could be changed without disturbing the suspended organ, the fresh solution being run in from a reservoir kept at the same temperature as the bath. The Ringer's solution was kept saturated with oxygen by bubbling the gas through it in a gentle stream. The uterus was suspended vertically between two platinum hooks, the lower one attached to the lower end of the oxygen tube, the upper one to a thread which hung from one end of a light lever, of which the other end bore the writing-point.

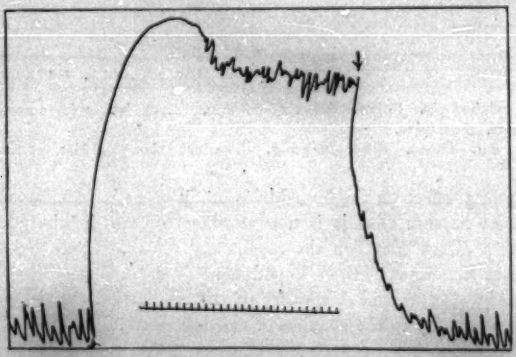

Fig. 15. Isolated uterus of virgin guinea-pig. Suspension method. Time 30". At † change to fresh Ringer. Upstroke = contraction.

Fig. 15 shows a record from the virgin uterus of a guinea-pig thus suspended. Left to itself in the warm Ringer's solution the organ quickly becomes completely relaxed, except for periodical small contractions. The figure shows the effect of adding to the bath 0·1 mgm. of β-I., of which the concentration in the bath was, therefore, 1 in 2,500,000. The uterus promptly contracts to its maximum tonus: after a time the originally smooth tonus becomes broken by a rhythm,

but the tonus remains high until the solution is changed, when relaxation quickly occurs.

Fig. 17 shows the effect in a similar experiment, of 0·01 mgm. of β-I., *i.e.* of a concentration of 1 : 25,000,000. The effect of 0·001 mgm., or 1 in 250,000,000, was still distinctly perceptible.

A quite similar tonic contraction of the uterus *in situ* was produced by the intravenous injection of somewhat larger doses. Fig. 16 shows the effect of one mgm. of β-I., injected intravenously, on the uterus of a pithed non-pregnant cat. The sense of the response is the same in all species and in all conditions as regards pregnancy. Quantitative differences of responsiveness occur, and it is somewhat remarkable that the most marked and persistent contraction with β-I. is obtained from the uterus of the virgin cat or guinea-pig; that is from an organ which responds to stimulation of its nerve supply or to adrenine by pure inhibition. The effect is quite unaltered by atropine. Late in pregnancy the sensitiveness of the uterus to β-I. is markedly diminished, as Kehrer observed with ergot extracts.

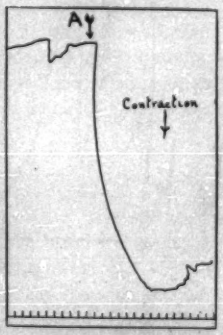

Fig. 16. Uterus of pithed cat *in situ*. Attached by thread and pulley to lever. Effect of 1·0 mgr. β-I. Down-stroke = contraction.

The action has no obvious relation to innervation, and, as might be expected, shows no coordination. The uterus contracts as a whole and tonically, except with minute doses. The tonic contractions thus directly produced by the drug can have no expulsive effect on the uterine contents, though it is probable that very small doses, by simply increasing the excitability of the muscle, would add power to the coordinate contractions produced by nerve-impulses or by the automatic rhythm of the organ.

Mention may be made at this point of the fact that preparations such as Witte's peptone, and various organ extracts, resemble β-I. in their action on the uterus as well as in other respects. Fig. 18 shows the effect on the isolated uterus of a virgin guinea-pig of adding to the bath 0·1 grm. of Witte's peptone, dissolved in warm Ringer's solution. The effect is indistinguishable from that of 0·1 mgm. of iminazolylethylamine. 0·5 c.c. of an extract of intestinal mucous membrane (containing also secretin) had a precisely similar effect.

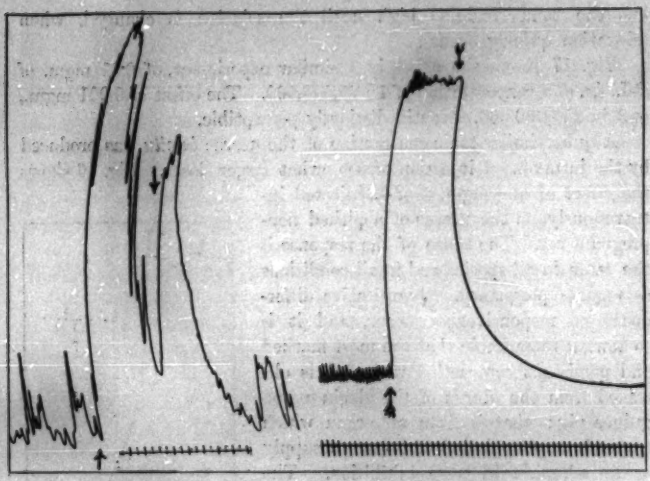

Fig. 17. Fig. 19.

Fig. 17. Similar to Fig. 15. Effect of 0.01 mgr. β-I.

Fig. 19. Isolated intestine of cat. Suspension method. At ↓ 0·5 mgr. β-I. added to 250 c.c. Ringer bath; at ↓ change to fresh Ringer.

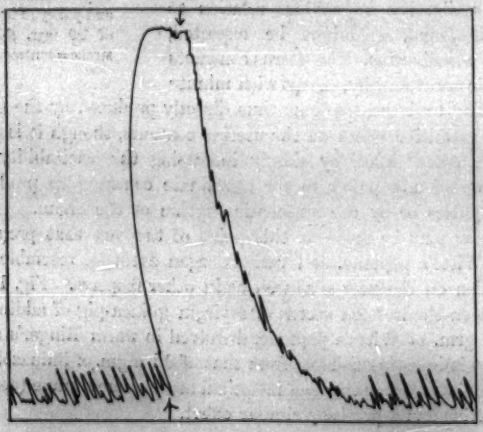

Fig. 18. As in Fig. 15. Effect of 0·1 grm. Witte's peptone.

The stomach and intestines.

The vomiting and purging produced in the unanæsthetised cat by intravenous injections of β-I. have already been mentioned. In the anæsthetised or pithed animal the effects on the exposed viscera of smaller injections (0·25—0·5 mgm.) are less striking, but still quite obvious. Both tone and rhythm appear to be augmented. An isolated loop of intestine, whether perfused artificially or simply suspended in warm oxygenated Ringer's solution, is more responsive to the stimulating action of the base than the undisturbed intestine with normal circulation. Fig. 19 (*supra*) shows the effect, on a short loop of cat's jejunum, suspended in the saline bath, of adding 0·5 mgm. β-I. to the bath, which had thus a concentration of 1 part of the base in 500,000. The effect is not abolished by atropine.

The urinary bladder.

In the intact animal the injection of β-I. frequently causes evacuation of the bladder. A record of the volume of the bladder contents in a pithed cat, by means of a catheter connected with a reservoir half filled with water, which again communicated with a bellows recorder, showed, as the result of a first injection of 0·25 mgm. β-I., a small relaxation of the bladder wall. Subsequent injections produced in every case a contraction of the bladder, the contractions becoming progressively larger with each injection, till the sixth injection produced a contraction leading to the expulsion of more than 40 c.c. of the contents. That this contraction was not due to direct effect on the musculature of the bladder was shown, however, by the fact that, after destruction of the sacral cord, a further injection caused practically no change of bladder volume.

We found little evidence, indeed, that the base has any marked direct effect on bladder muscle. All the effects which we observed can fairly be attributed to the stimulation of centres in the cord by the anæmia caused by fall of arterial pressure.

The plain muscle of other organs.

The retractor penis of the dog or goat is thrown into tonic contraction by small doses of β-I. Good responses were obtained, when the muscle was treated as an isolated organ, with dilutions of 1 in 250,000.

In the anæsthetised animal no effect was observed on the size of the pupil as the result of injecting intravenously 0·25 to 0·5 mgr. β-I.; but in unanæsthetised cats, receiving larger doses by intravenous or hypodermic injection, constriction of the pupil, even in the dusk, is a noticeable feature of the effect, as already mentioned. It may be doubted whether this is in any degree due to direct effect on the plain muscle of the sphincter. The fact that the pupil, though remaining small when the eyes are shaded, dilates when the animal is roused or excited in any way, seems rather to indicate that the constriction is of central origin and associated with the narcotic action of the base. We have seen no evidence of an effect of the base on the pilomotor muscles.

Gland cells.

Salivary gland. Salivation has been mentioned as one of the effects of the base on the unanæsthetised cat. Experiments on the anæsthetised cat and dog show that this effect is, in part at least, due to a direct peripheral action of the drug. In a dog anæsthetised with A.C.E. mixture a cannula was inserted into Wharton's duct and connected to a narrow horizontal glass tube attached to a millimetre scale. The chorda tympani was cut and stimulated with a weak tetanising current for a few seconds. When the flow of saliva resulting from the preliminary stimulation had ceased an intravenous injection of 0·25 mgm. β-I. was made. A flow of saliva began almost at once, rapidly reached its maximum rate and then quickly declined. The total movement of the column of fluid along the tube, resulting from the injection, was 8 cm., representing about 0·55 c.c. saliva. A second injection of 0·5 mgm. caused the secretion of about 0·7 c.c. saliva. 1 mgm. of atropine was administered intravenously. A further injection of 0·5 mgm. β-I. then produced no trace of salivary secretion. A very similar effect was produced in a cat anæsthetised with paraldehyde.

A secretion of tears can usually be observed as a result of an injection of β-I. in the anæsthetised cat.

The part probably played by increased bronchial secretion in the respiratory impediment produced by the base has already been mentioned.

The pancreas. β-I. has also a definite stimulant effect on the pancreatic secretion. In a dog anæsthetised with A.C.E. mixture a cannula was inserted into the pancreatic duct and the flow of pancreatic juice recorded by a drop recorder. 10 c.c. "secretin" (acid duodenal extract) were injected intravenously and 135 drops of juice were secreted in the

16 minutes following the injection, at the end of which time the flow had returned to its original rate of about four drops in 10 minutes. 1 mgm. β-I. was then given, the cannula being first carefully freed from secretin, and 25 drops of juice were secreted in the 10 minutes following the injection, the first 20 drops being secreted in the first five minutes of this period. 5 mgms. of atropine were then given and after a short interval 1 mgm. β-I. was administered intravenously. Four drops of juice were secreted in the 10 mins. following the injection: that is to say, the rate of secretion was not affected at all. 10 c.c. of secretin were then given and over 90 drops of pancreatic juice were secreted in the seven mins. following the injection, the experiment being discontinued while rapid secretion was still in progress. It is thus evident that β-I. evokes a small secretion of pancreatic juice, but that this action is completely abolished by atropine, which leaves the effect of secretin unchanged. In other directions the injection of 10 c.c. of the duodenal extract was roughly the physiological equivalent of that of 1 mgm. of β-I. Each produced a fall of blood-pressure from 110 mm. to about 25 mm., the recovery being rather slower after the 1 mgm. of β-I. We shall mention later certain considerations which suggest the possibility that the duodenal extract may contain β-I. or a closely related substance, which would account for some at least of its depressor action. However that may be, it is abundantly clear that the slight action of β-I. on pancreatic secretion is of a different type from, and of an altogether lower order of intensity than, that of secretin.

Kidney. We found no evidence that β-I. had any effect on renal secretion apart from that produced by vascular changes. The rate of urinary flow followed with tolerable accuracy the arterial blood-pressure.

Excretion. β-I. is not excreted as such in the urine. The urine of the cat to which 150 mgms. in all were administered hypodermically was collected during the 48 hours succeeding the first injection. It gave Pauly's reaction with diazobenzenesulphonic acid with great intensity, but had no perceptible effect on the isolated uterus of a guinea-pig in doses of 5 c.c. This seems to point to excretion as a compound devoid of the characteristic physiological activity, but with the iminazole ring intact. This compound has not as yet been identified.

Striped muscle.

The effect of β-I. on striped muscle is altogether insignificant as compared with its effect on the plain muscle of most viscera. The application of a 1 in 1000 solution of the hydrochloride in 6 % saline to a muscle-nerve preparation, produced no contraction. The preparation was left overnight in the solution and the muscle then responded to the excitation of its nerve with a single induction shock by a twitch, which differed from that given by a control preparation (from the other leg) kept in saline for the same time, only in being slightly lower. The effect may be regarded as practically nil.

Discussion and Summary.

The action of β-iminazolylethylamine, as described in detail in the preceding sections, appears a somewhat complicated one. It cannot be summarised with reference to any division of the autonomic system, like that of some other amines. The fundamental and characteristic feature of its action is its direct stimulant effect on plain muscle, in which it produces exaggeration of rhythm with increased tonus, or steady maximal tonus unbroken by rhythm, according to the concentration in which it is applied. The sensitiveness of plain muscle from different organs and in different species varies within wide limits. The most sensitive of all appears to be the plain muscle of the uterus: the non-pregnant uterus of some species responds to the drug in extreme dilution. The muscular coats of the bronchioles are also highly sensitive to the action, especially in rodents. The plain muscle of the intestinal wall, of the arterioles and of the spleen appears to occupy an intermediate position as regards responsiveness: that of the bladder and the iris was not perceptibly affected by the direct action of such doses as we employed. Cardiac muscle is mildly stimulated by the drug: skeletal muscle not perceptibly affected in any way.

In rodents the main features of the effect are manifestations of this action on plain muscle. A rise of blood-pressure is produced in these animals by constriction of the arterioles, unless this is masked by embarrassed respiration due to bronchial constriction, which in its severest form causes death by asphyxia. The only additional effect to be noted in the rodents is a narcotic action, especially manifest with large doses given hypodermically.

In carnivora the direct action on plain muscle is overcome, in the case of the systemic arterioles, by an antagonistic peripheral action, the mechanism of which is not clear. This result is general vaso-dilatation, in which the kidney vessels, however, do not participate, causing a fall of systemic blood-pressure. The pulmonary arterioles on the other hand constrict in response to the drug whether in the body or isolated. Thus β-I. produces in carnivora the association of a rise in pulmonary with a fall of systemic pressure, described by Bradford and Dean[1] as characteristic of ergot alone among the drugs with which they experimented. Narcosis is also observed in carnivora, and the constricted pupil may be regarded as a feature of this narcotic effect, since it is not produced by β-I. in animals otherwise anæsthetised. In addition the base has a mild direct stimulant action on the activity of the salivary glands and the pancreas. This secretory effect, being paralysed by atropine, may be regarded as a weak action of the pilocarpine type: the association has some interest in that pilocarpine also contains an iminazole ring. The action on the pancreas is not at all like that of secretin.

In the case of several features of the action of β-I. we have drawn attention to the similarity of its effect to that of Witte's peptone and of certain organ extracts. It has been recognised that a certain symptom-complex is common to the action of extracts from a number of organs, and Popielski has suggested the name "vasodilatin" for a hypothetical depressor active principle, present in such extracts and in preparations such as some commercial "peptones," and responsible for the effects which they have in common. It is of interest to note that this common group of effects, in almost all essential particulars, is produced by β-I. We would add to the common actions, described by Popielski and others, the direct stimulant action on plain muscle, as exemplified by the action on the isolated uterus. In one respect the parallelism appears to break down. In the few experiments as yet made for the purpose, we have been unable to detect any action of injections of iminazolylethylamine on the coagulability of the blood. In other respects, however, the correspondence in action is so close as to suggest the presence in "peptone" and in various organ extracts of some substance at least related to iminazolylethylamine. The matter is at present the subject of chemical investigation by Dr Barger[2].

[1] *loc. cit.*

[2] Since the above was written β-iminazolylethylamine has been isolated from intestinal extract. A forthcoming paper by Barger and Dale will give the details.

This symptom-complex has recently acquired interest in another direction. Biedl and Kraus[1] drew attention to the identity of the symptoms of anaphylactic shock with those produced by intravenous injection of "peptone," which, as we have seen, are again very largely identical with those of β-I. The correspondence cannot yet be regarded as sufficient basis for theoretical speculation, and we content ourselves with recording, as a point of interest and possible significance, the fact that the immediate symptoms with which an animal responds to an injection of a normally inert protein, to which it has been sensitized, are to a large extent those of poisoning by β-iminazolylethylamine.

[1] *loc. cit.*

CONTRIBUTIONS TO PHYSIOLOGICAL CLIMATOLOGY. PART I. THE RELATION OF LOSS OF WATER FROM THE SKIN AND LUNGS TO THE EXTERNAL TEMPERATURE IN ACTUAL CLIMATIC CONDITIONS.

By W. A. OSBORNE.

(*From the Physiological Laboratory, University of Melbourne.*)

The reaction of the animal body to varying temperatures of its environment and to air of varying humidity has already been the subject of extended investigations. In by far the greater number of these observations the temperature and humidity were artificially produced. From the standpoint of climatology such experiments afford valuable indications and clues but do not constitute proof of how the animal body will react when subjected to true climatic conditions. It was the object of this inquiry to make observations under more normal conditions.

The subjects of the research were W. A. O., aged 36½ years, weight 68 kilos, and A. C., a robust country lad, aged 14 years, weight 46 kilos. The experiments were carried out at Warrandyte some eighteen miles inland from Melbourne. The altitude was about 150 feet above sea level. The site was close to the river Yarra and the adjacent country was undulating and well timbered. A clump of pine trees afforded perfect shade during the hours of experiment. Wet and dry-bulb shade temperatures were taken by Centigrade instruments checked before and after summer in the Melbourne laboratory. In the case of the wet-bulb thermometer it was found inadvisable to depend on the supply of water brought by a wick from a reservoir. The bulb was jacketed with thin cotton stuff and made wet by immersion in a beaker of roof rain water. On removing the beaker the temperature fell and the minimum was taken as the W. B. reading. In addition a wet and dry-bulb recording thermograph made by Richard Frères was employed. The dry-bulb readings were fairly free from error but the wet-bulb readings were

unreliable. When, however, the wet-bulb lever was adjusted to give the correct W. B. reading, its record could be depended on for a couple of hours provided no great change in D. B. temperature had occurred in the meantime. These thermographic records were found of great value in fixing a mean D. B. and W. B. temperature when these, in a few cases, varied during the hour taken up by the experiment. The barometric pressure was taken with a pocket aneroid (Stanley) checked in December and March in the Melbourne laboratory. The subject was weighed before and after an hour's rest in a hammock by a "human balance" made by De Grave, Short and Co. The weighings were taken to the nearest gram. The balance was kept in a cool room in a wooden house about twenty yards from the hammock. The volume of air expired in a given time was registered by a Zuntz portable respiration meter (latest pattern). The air in the sampling pipette was analysed by a modification of the Hempel method. The burette was surrounded by a water jacket in which a thermobarometer of the Zuntz pattern was sunk. Acidulated water was used as the enclosing fluid. Carbon dioxide was absorbed by sodium hydrate solution and oxygen by wet phosphorus. In the case of W. A. O. the clothing varied with the weather but was limited to certain fixed types given in detail below. The object here was to choose just that clothing which was comfortable at the time. With A. C. however the clothing was the same in all experiments, namely a light shirt, knickerbocker suit, light socks and slippers[1].

The detail of an experiment with myself may be given as follows. The first weighing was timed to finish at 11.30 a.m. This was about three hours after the conclusion of breakfast, the interval being taken up with reading and work of a light description. The breakfasts were practically uniform in character. On the few days when two experiments were carried out the second hour chosen was from 2.30 to 3.30 p.m. Before the first weighing the thermograph was adjusted and the barometer and thermometers read. As soon as the weighing was finished and the balance left ready for a second weighing I lay down on an open-mesh wire hammock for one hour remaining as motionless as possible. Close beside the hammock was a stand on which was placed the Zuntz meter ready for use. When about half an hour had elapsed the meter was breathed into for ten minutes, the spindle being chosen so that the sample vessel should be well emptied of its acidulated

[1] The slippers worn by both subjects were discarded before weighing to avoid errors arising from earth etc. lost or gained in the short walk between hammock and balance.

water in that time[1]. The temperature of the expired air circulating through the meter was noted. At the end of the hour I weighed myself quickly and then proceeded to examine the instruments and do the gas analyses. In the case of A. C. the hour chosen was from 2.30 to 3.30 p.m., all the readings etc. being taken by myself. In both cases adequate precautions were taken to avoid any vitiation of the results by errors due to micturition, expectoration or alteration in the contents of the pockets etc., and no experiments were conducted during rain. In hot weather I took my rectal temperature but never found any departure from normal. The importance of anemometric readings was not realised until the experiments were concluded—in future work these will be taken.

The data obtained in each experiment by the above methods were as follows: (*a*) the air temperature, (*b*) the wet-bulb reading, (*c*) the barometric reading, (*d*) the weight loss in one hour, (*e*) the volume of air at temperature t expired in ten minutes, this figure multiplied by 6 gave the volume per hour, (*f*) the per cent. by volume of CO_2 and O_2 in the expired air.

From the wet-bulb reading and from the difference between the wet and dry-bulb readings the dew-point was calculated in the usual manner. From the volume of air expired at pressure p and temperature t the volumes were calculated for standard pressure and the following temperatures: (*a*) 0° C., (*b*) dew-point, (*c*) 37° C.

Now the air at dew-point would be saturated with water vapour, hence the mass of water in the inspired air could be obtained from tables = *W.* insp., if we make the simplifying assumption that the volume of air expired is equal to that inspired at the same temperature and pressure. Now the air leaving the lungs may be taken to be saturated with water vapour and to have a temperature of 37° C. Hence from tables the mass of water in the expired air could be obtained = *W.* exp. Therefore the mass of water given off from the body through the lungs = *W.* exp. − *W.* insp.

As certain simplifying assumptions are made here, and as some doubt will always exist as to the constant to be employed in calculating the dew-point, the values for the lung water-loss can only be regarded as approximate. The volume of air expired was reduced to 0° C. and standard pressure to simplify the calculation of the mass of CO_2 which it contained and of the mass of O_2 absorbed. In the case of the CO_2 a

[1] Breathing proceeded about one minute before I pulled the lever that started the fall of the sample leveller.

TABLE.

Experiments with W. A. O. *Rest in shade. The quantitative data are for one hour. Weights are expressed in grams; volume of air expired in litres, temperature by Centigrade scale.*

Date 1910		Dry bulb	Wet bulb	Weight loss	Total water loss	Lung water loss	Air expired at temperature	CO_2 expired in grams	Respiratory quotient	Costume	Remarks
Jan.	6	34	20	230	—	—	—	—	—	D	
	6	37·2	20	254	—	—	—	—	—	E	
	8	36·1	21·1	284	—	—	—	—	—	E	
	9	28·9	20·5	108	—	—	—	—	—	D	
	9	27·2	20·5	106	—	—	—	—	—	D	
	11	20·2	15	62	57·1	14·2	393·7 25°	23·63	0·93	B	
	15	21·1	17·6	44	39·5	13·3	400 21·1°	22·56	0·92	B	
	17	17·3	13·6	50	46·5	14·5	390 18·3°	21·62	0·86	A	
	19	27	18	107	101·5	15·5	444 20°	23·99	0·95	C	
	20	21	14·5	88	85·4	13·4	354·5 21°	18·36	0·86	B	
	21	18·9	14·6	62	57·8	14·4	392·4 18·9°	19·9	0·93	B	
	23	20·2	14·4	79	76	13·9	370 20·2°	18·75	0·87	B	
	24	29·2	17	152	147·4	14·9	420 32°	22·37	0·93	D	
	25	35·8	20	362	357·4	17·1	471 36·5°	25·05	0·93	E	Strong N. breeze.
	25	39·4	21·1	323	—	—	—	—	—	E	Breeze moderated.
	26	27·2	20	112	106·4	15	465 29°	25·47	0·95	D	

Jan.	30	25	14·6	87	83	16·1	410 25°	22·8	0·89	C	
	31	27·2	16·75	107	102·5	15·8	432 29°	21·17	0·93	D	
Feb.	2	33·6	18·1	235	230·2	16·85	441·6 35°	21·29	0·95	E	Fair N. wind.
	3	26·1	17·7	95	91	14	395 27°	21·39	0·95	C	
	4	21·1	13	68	64·1	14·67	395 22°	21·75	0·9	B	Cool, dry wind, skin feeling dry.
	6	23·3	13·8	72	—	—	—	—	—	B	Fitful light wind, skin feeling dry.
	8	21·7	15	89	85	14·7	395 22°	21·26	0·91	C	
	12	19	12·6	98	94·4	17·8	438 20°	22·32	0·88	A	Fair S.E. wind, skin feeling dry.
	13	21·7	12·5	98	94·8	17·5	432 21·1°	23·78	0·83	C	S.E. wind, skin feeling dry.
	15	31·7	16·5	157	150·6	20	504 33°	24·38	1	E	Calm.
	16	32·8	18·8	252	247·4	18·2	498 35°	21·85	0·99	E	N. wind.
	16	35	19·1	275	—	—	—	—	—	E	N. wind.
	18	22·5	18·2	80	76·6	13·5	412·5 23°	21·07	0·7	B	Calm.
	19	37	20	270	262·6	21·8	589·8 35°	28·96	1	D	
	25	19·3	14·6	67·5	62·6	14·9	405 22·5°	22·75	0·93	B	Fresh S. breeze.
March	5	23·9	15	76	71·4	16	416·4 23·9°	22·7	0·92	C	Slight N.W. wind.
	12	17·5	13·9	74	69·2	15·6	426 19°	24·14	0·92	B	Slight S. wind.
April	3	15	11·6	60	56·5	16·24	854 21°	22·3	0·87	C	

TABLE (*continued*).

Experiments with A. C. *Rest in shade.*

Date 1910	Dry bulb	Wet bulb	Weight loss	Total water loss	Lung water loss	Air expired at temperature	CO_2 expired in grams	Respiratory quotient	Costume	Remarks
Jan. 25	38·3	21·1	212·3	208	18·5	480 38°	25·36	0·88		
Feb. 2	35·7	19·7	181	174·5	15·4	417·6 35°	25	0·99		
15	23·9	13·2	55	51·3	17·2	420 24°	22·45	0·88		
25	22·8	16·3	61	56·2	13·1	421·8 22·8°	23	0·9		
March 12	17	13·5	47	43·5	14·7	396 17°	22·1	0·87		
22	30·5	17·8	123	—	—	—	—	—		

Experiments with W. A. O. *Walking in shade.*

Feb. 11	21·1	14·7	63	57·2	22	590 22°	32·4	0·9	C	Cool, slight S. wind.
13	21·1	13·1	112	108·6	26·69	685 24°	37·3	0·81	C	Easterly wind, skin feeling dry.
13	21·1	13·2	—	—	—	1104 21°	—	—	C	Walking briskly.

Experiment with W. A. O. *Work (chopping logs).*

15	32·8	16·4	840	—	—	—	—	—	E	North wind.

small correction was made for the CO_2 in the inspired air which was taken as 0·04 %. The percentage of O_2 present was subtracted from 20·6 %—the figure given by my instrument for the oxygen percentage of ordinary air saturated with water vapour. This gave the percentage of O_2 absorbed and hence the mass. The respiratory quotient was obtained in the usual way. The actual water-loss of the body during the hour was obtained by adding to the weight loss given by the balance the weight of oxygen absorbed and subtracting the weight of carbon dioxide given out.

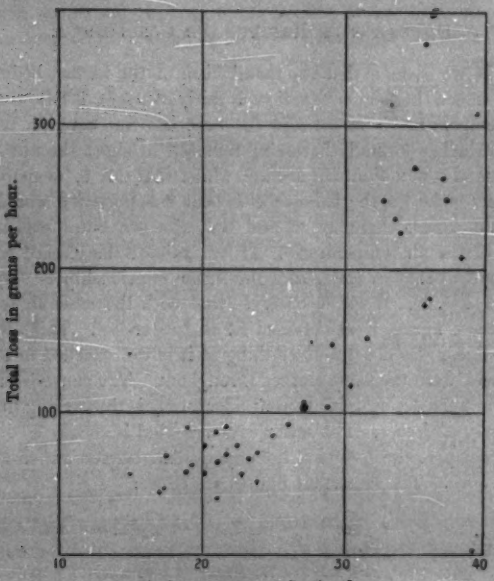

Fig. 1.

Results. These can best be shown in tabular form. Some results of walking experiments and one of work are also given. The costumes worn by W. A. O., and denoted in the penultimate column by a letter, were as follows:

A. Flannel shirt; serge trousers, waistcoat and jacket; thick socks.

B. Flannel shirt; serge trousers and waistcoat; light socks.
C. Flannel shirt; serge trousers; light socks.
D. Serge trousers; light cotton shirt; light socks.
E. Drill trousers; light cotton shirt; light socks.

Appended is also a graphic representation of the relation between the total water-loss and the air temperature. In the few cases in which the weight loss alone was obtained by experiment I have subtracted 5 grams as an approximate correction. The circles denote results with W. A. O.; the crosses those with A. C.

Discussion of Results and Conclusions.

(1) If we assume that the metabolism of the human body during rest, and hence the heat production, is fairly constant, it follows that, as the air temperature is raised and the heat interval between body and air diminished (eventually becoming reversed in sign), the heat-loss by radiation and conduction will decline, whilst that due to evaporation of water must make up the balance, else heat accumulation would occur. One would expect therefore to find that the water-loss was a linear function of the air temperature[1]. If we examine the plotted results it will be evident that in both series the linear relationship is very much perturbed (Fig. 1). It will be observed that, with the same air temperature, different figures were obtained for the water-loss. This departure from the linear was not due to clothing as it is well marked in the case of A. C. who wore the same clothes throughout. The cause is probably complex but one determining factor undoubtedly is the varying humidity and velocity of the air. The action of a dry wind is clearly brought out when we compare the results of the experiments carried out on Feb. 8th and Feb. 13th. The important figures are these:

	Dry bulb	Wet bulb	Water-loss in grams	CO_2 given out per hour in grams
Feb. 8th	21·7°	15°	85	21·26
,, 13th	21·7°	12·5°	94·8	25·78

These figures taken in conjunction with those of Jan. 15th and Feb. 4th and other dates show clearly that the air temperature may be the same but, if the relative humidity fall, the water-loss is increased.

[1] The statement found in so many text-books that the heat-loss by radiation and conduction makes up the greater portion of the total heat-loss is true only of certain climates and weather conditions. An air temperature equal to that of the body will reduce this loss to zero.

That this should occur at temperatures well below that of the body is a remarkable fact to which little attention has been paid. The increased water-loss will assuredly mean an increased heat-loss and therefore an increased metabolism in the body if the latter is to remain thermostatic. The problem may be perhaps put in the following form. If the air is dry and in movement it will tend to desiccate the skin. But there must be a limit to the extent of desiccation as the skin can easily be injured, owing to the fact that many of its physical properties depend on the water of imbibition which it contains. If, however, the normal state of imbibition of the skin be maintained by vaso-dilatation of the peripheral vessels and by perspiration, the heat-loss will become excessive if the air temperature is well below that of the body. In such conditions if the body is to preserve its constant temperature with constant metabolism the skin will be injured. If body temperature and skin imbibition are to be maintained constant then the metabolism must be augmented. What apparently occurs is a compromise between the two extremes; the skin loses some of its water of imbibition and the metabolism undergoes a moderate rise. This increased demand on the metabolism of the body when the wind is cold, dry and in movement, may be one of the causes of the proverbial unpopularity of the east wind in north-west Europe.

(2) Another fact brought out clearly in these experiments is that the ventilation of the lung is markedly increased when the air temperature is high. To dwellers in a climate where the summer shade temperature rises above 100° F. this fact is obvious, at least as regards the lower animals. I have frequently noted respiration rates of 300 per minute and over in the domestic fowl with the thermometer reading 100° to 104° F. in the shade. To the list of agents which excite the respiratory centre we must add a high environmental temperature. In what manner this acts I cannot at present surmise for on those hot days in which my lung ventilation, as measured by the Zuntz meter, was increased I did not detect any rise above normal in my rectal temperature provided I remained at rest[1]. That the arterial blood leaving the lung could have a higher temperature than the body is scarcely possible if we consider the heat-loss through pulmonary evaporation. The only conjecture that I can make is that the carotid blood may become heated in its passage up the neck.

[1] Obviously when the air temperature is higher than that of the body the reading of the clinical thermometer must be done quickly. Fortunately the moisture adhering to the bulb prevents the thermometer taking up the air temperature at once and gives time for a reading.

(3) It will be observed that contrary to the usual reaction of the homoiothermal animal the carbon dioxide expired varied directly and not inversely as the external temperature. This is confirmatory of some results of Voit[1] and of what Rubner[2] found to be the case with the human subject when clothed. As it is manifest in the experiments with A. C. who retained the same clothing throughout some further explanation than clothing must be sought. It is significant that the ventilation of the lung also varies directly as the external temperature so that the additional work of the respiratory muscles in itself would cause an increase of CO_2 output. Perhaps also "washing out" of CO_2 from the tissues plays some part here.

(4) There is some indication that with rising shade temperatures (wet and dry-bulb) the respiratory quotient rises and approaches unity. This is interesting in view of the experiments of Dr Harvey Sutton[3] who found that the respiratory quotient approached unity when the wet-bulb temperature rose in a room which he could artificially make warm and moist. Dr Harvey Sutton has made some interesting suggestions regarding the relation of this reaction to the preponderance of carbohydrates in the diets of tropic aboriginals.

The expenses of this research were defrayed by a grant from the Government Grant Committee of the Royal Society.

[1] *Zeit. f. Biol.* XIV. p. 78.
[2] *Biologische Gesetze*, Marburg, p. 30. 1887.
[3] *Journ. Path. and Bact.* XIII. p. 62. 1908.

THE INFLUENCE OF LACTIC ACID UPON THE DISSOCIATION CURVE OF BLOOD.

By JOSEPH BARCROFT, F.R.S., *Fellow of King's College, Cambridge,* AND L. ORBELI, M.D. (*St Petersburg*).

(*From the Physiological Laboratory, Cambridge.*)

BOHR[1] has shown that carbonic acid has a remarkable effect in altering the affinity of blood for oxygen. More recently, Barcroft and Camis[2] have observed that the affinity of a hæmoglobin solution for oxygen depends upon the inorganic salts present in the solution. We are then confronted with the question, Is the effect of carbonic acid upon the dissociation curve of blood a specific effect, or is it merely a special case of the general ionic effect observed by Barcroft and Camis? In the present paper it will be shown that at least one other organic acid, lactic acid, can act in a similar way to carbonic acid and that this action has a certain physiological significance.

The determination of the dissociation curves which are given below, have been made by a method not as yet described.

Whether the subject of the experiment be a small animal (whose curve it is desirable to study from time to time) or whether it be a human being, it is clear that no method can be satisfactory which involves the abstraction of more than one or two cubic centimetres of blood at a time. On this quantity of blood therefore the curve must be determined.

Moreover a source of great inconvenience in the method described by Barcroft and Camis is, as mentioned by them, the fact that the amount of carbonic acid which it is necessary to introduce into one of the tubes in order to give a specified tension depends upon unknown factors in the constitution of the blood. These unknown factors determined how much CO_2 the blood took from the atmosphere or gave to it.

This trouble can be avoided if the quantity of blood used is so small relatively to the atmosphere to which it was exposed that any changes which take place in the gaseous content of the blood may be considered as not affecting the constitution of the atmosphere.

By the following method six or seven points may easily be determined from 1·5 c.c. of the blood of the animal. It is necessary to have a similar amount of defibrinated blood, this may come from any animal.

A tonometer of about 250 c.c. is used. It consists of a glass bulb of the form shown in Fig. 1. At one end the tonometer is closed with a rubber cork, at the other it connects by a three-way tap with what is really a delivering pipette. The tubing in connection with this tap should not be more than 2 mm. bore. The extremity of the pipette must be drawn out carefully to the required dimensions. These dimensions are regulated by the following considerations. (1) The tip must reach to the bottom of every blood gas bottle which is used, and therefore for the whole length of one of these bottles (about 5 cm.); the outer diameter of the pipette must not exceed the bore of the bottle neck. (2) The bore of the pipette must not be so fine that the blood, even when mixed with droplets of mercury, will not run out. For reasons which will later appear, the first portions of fluid which come out of the tonometer consist of fine globules of mercury mixed with a little blood. Such a mixture is not easy to drive along a tube of fine and gradually diminishing bore and we have had recourse on occasions to the expedient of placing the bent tube above the tap in connection with a cylinder of compressed gas for the purpose of clearing the pipette. With a properly constructed pipette such expedients are not required. (3) The volume of blood delivered should be approximately $\frac{1}{10}$ c.c.

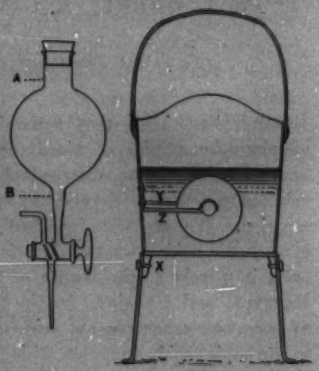

Fig. 1.

Fig. 2.

The tonometer must of course be absolutely dry to start with. It is then filled with mercury and nitrogen is introduced through the

pipette till the mercury has all been displaced. The tonometer is then corked up.

The nitrogen which we have used in the present research has been supplied to us in cylinders. Such nitrogen must be carefully analysed, and especially tested for the presence of carbon monoxide. The only method which we can regard as satisfactory for this purpose is to make two series of determinations of a point in the dissociation curve of the same blood. In the first series nitrogen obtained by abstraction of oxygen from atmospheric air should be used, in the second the nitrogen which is the subject of the test. We shall have more to say on this subject in a future paper. Here we will confine ourselves to the statement that an amount of carbon monoxide which is quite inappreciable in its effect when competing with oxygen at the atmospheric pressure for hæmoglobin will upset the parts of the dissociation curve at which the pressure of oxygen is very low. The point on the curve chosen for investigation should be at somewhere about 20 mm. oxygen tension.

When the tonometer has been filled with nitrogen a calculated quantity of carbonic acid is put into it from a gas burette; the amount should be more by about 4 % of its volume than is necessary to simulate the alveolar CO_2 tension or whatever tension may be required. This slight surplus is used up in the analysis of a sample of gas which is withdrawn into a Haldane's gas analysis apparatus at this stage. In this sample the oxygen, nitrogen and carbonic acid are measured, and a note is made of the pressure of the residual gas in the apparatus. It is desirable that this pressure should be appreciable, commonly it is about 20 mm. of mercury. The positive pressure is easily measured by comparing the surface of the mercury in the reservoir of the gas analysis apparatus with the meniscus in the stem of the gas pipette. The reading is taken in graduations of the stem and translated into millimetres.

The blood is now put into the tonometer. It is forced through the pipette by a hypodermic syringe and is washed in with a little mercury, which is left to fill up the dead spaces.

The tonometer is placed in a bath (Fig. 2) which stands on legs screwed on to the rim. From the inside of the bath project two arms *Y* and *Z* each with a hook which fits the tonometer. When the bath is filled with water the tonometer tends to rise but is prevented from doing so by the hooks which grip it at *A* and *B* (Fig. 1). A cord is passed once or twice round the neck of the tonometer; when the ends of this

are pulled alternately the tonometer is rotated in the bath and the blood is spread out into a large film over the surface of the glass. The temperature of the bath in the experiments which follow was 38° C.

About ten minutes rotation is sufficient to get the blood into equilibrium with the gas. The tonometer is then released from the hooks and whilst still under water is placed in a vertical position, as nearly as may be. The blood collects in the vicinity of *B* (Fig. 1) and a small surface only is exposed. The tonometer is taken out of the bath; the tap is opened; the pressure inside the apparatus forces out first the mercury and then the blood: the former, followed perhaps by a drop of blood, is allowed to make its way out. It will be found most convenient to make the orifice of the pipette rest upon a cloth. The rate at which the fluids in the dead space escape can then be easily regulated. If there is any trace of grease in the pipette, as may easily happen, it is best to fill it with mercury, to the exclusion of all air, before allowing any fluid to escape from the tonometer, otherwise minute bubbles of air may adhere to the glass and affect the blood. The pipette is now full of blood, the tap is turned through 90° and the orifice of the pipette is placed under the ammonia solution of a blood gas bottle. On turning the tap through a further 90° the blood is delivered into the ammonia without contamination with air.

A calculated quantity of oxygen is then forced into the tonometer from a gas pipette and the cycle of operations is repeated, thus point after point on the curve is determined.

As it has been assumed that the changes produced by the action of the blood upon the atmosphere may be neglected, an analysis of the residual gas in the apparatus made at the end of the experiment should agree with the result given by the initial analysis and the quantities of oxygen successively introduced. (It is of course necessary to know the degree of purity of the oxygen.) The method has proved satisfactory in this respect, the two calculations agreeing generally to within a millimetre pressure of oxygen. In making the final calculation it must not be forgotten that the pressure in the bulb is reduced by the abstraction of the gas for analysis. This pressure can be measured on the stem of the gas analysis pipette.

By this method the numerous gas analyses of the method used by Barcroft and Camis are reduced to two.

The blood gas apparatus used is that form of differential apparatus described for the analysis of 0·1 c.c. of blood by Barcroft and Roberts[20].

We may now consider the accuracy of the method. The following is a series of consecutive analyses of sheep's blood at different tensions. The figures given are the percentage saturation with oxygen.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
4 %	23 %	24 %	27 %	44 %	48 %	66 %	67 %	80 %
2	18	24	28	46	49	66	73	80
2·5	18·5	25	31			70		

The range of variation is 6 %; it may therefore be taken that any particular analysis is within about 3 % of the correct figure.

In the case of cat's blood, in which there is less hæmoglobin than in sheep's blood, the error appears proportionately larger.

(1)	(2)	(3)	(4)	(5)	(6)	(7)
13 %	15 %	36 %	65 %	71 %	78 %	89 %
17	19	43	67	76	79	90

For human blood the following duplicates may be given.

(1)	(2)	(3)	(4)	(5)
78 %	82 %	61 %	77 %	83 %
79	80	62	79	84

The following is an instance where the present method was tested against the older one; at a tension of 20 mm. of oxygen the former gave a saturation of 27·5 %, the latter 30 %. In another case blood was analysed in the apparatus for 1 c.c. and gave 88·7 %, the same blood in two of the small apparatus gave 88 % and 89 % respectively.

The gas analysis apparatus used was the portable type described by Haldane.

Results obtained with defibrinated blood.

The first experiments which we performed were with defibrinated sheep's blood. We started with the normal blood, as brought from the butcher and strained through muslin. The following points were determined.

T[1]	26 mm.		47 mm.	67 mm.	89 mm.
S	29 30 33 } mean 31 %		68 72 68 } mean 70 %	87 %	94 %

[1] Throughout T = tension of oxygen in mm. of mercury. S = percentage saturation of blood with oxygen.

These points, two of which appear as the mean of three determinations each, are very close to the curve given by Barcroft and Camis (Fig. 4, p. 126) for sheep's blood at 40 mm.[1] CO_2 pressure (the pressure at which all the determinations in the present paper have been made). For the same oxygen tensions as the above the following are the percentage saturations given in the former research:

T	26 mm.	47 mm.	67 mm.	89 mm.
S	80 %	74 %	87 %	94 %

It seems clear that this curve is characteristic for sheep's blood, for oxygen tensions above 25 mm. of mercury.

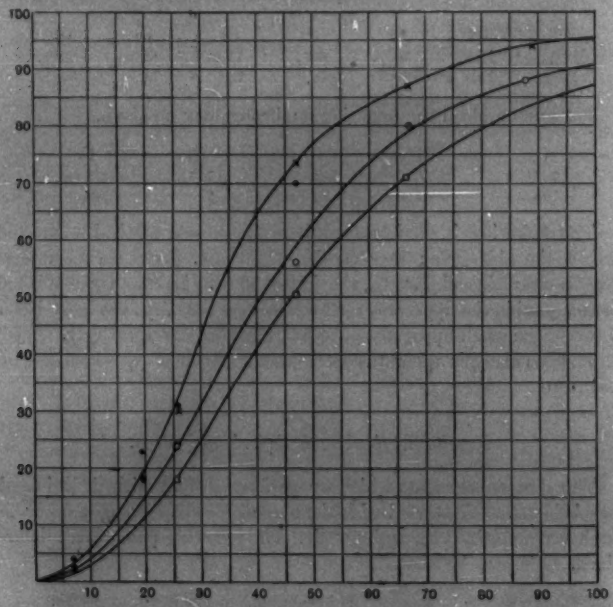

Fig. 3. Dissociation curve of sheep's blood. • normal blood. × normal blood, that to which lactic acid was added for subsequent determinations. ○ contains 0·04 % added lactic acid. □ contains 0·08 % added lactic acid.

[1] This opportunity may be taken of correcting a typographical error in the figure referred to. For the Roman numerals I—V in the figure, read V—I.

The method employed by Barcroft and Camis was very inconvenient for determinations below 20—25 mm. oxygen tension. In the small tubes used, the blood gave out sufficient oxygen to establish a tension of this order if placed with commercial nitrogen. It was therefore not quite certain what the curve was like at very low tensions. With the present method there is no difficulty in getting down to 5—7 mm. oxygen tension. We have therefore made three careful determinations at 7 mm. tension and three more at 19·5 mm. with the following results:

T	7 mm.	19·5 mm.
S	2, 2·5, 4, mean 3 %	18, 18·5, 23, mean 20 %

For the purpose of testing the effect of acid we added 1 c.c. of the B. P. solution of lactic acid (which contains approximately 75 % of the acid) to 100 c.c. of blood. The lactic acid was diluted in about 3 c.c. of Ringer's solution and added little by little to the blood which was kept in a constant state of agitation. The following results were obtained:

T	25 mm.	47·5 mm.
S	14 %	45 %

Clearly the affinity of the blood for oxygen was very much reduced. The amount of acid which had been added was too great for the experiment to be regarded as more than of a preliminary nature. The blood became acid to litmus.

In our next experiment we added 0·1 c.c. of lactic acid solution to 100 c.c. of blood and obtained the following results:

T	25·5 mm.	47 mm.	66·5 mm.
S	18 %	50·5 %	71 %

When these figures (indicated by the squares on Fig. 3) are compared with those from normal blood it is clear that the dissociation curve is very much depressed.

We mixed equal parts of the blood used for the above experiments and normal sheep's blood so that the 100 c.c. of the mixture contained 0·05 c.c. of added lactic acid or just under 0·04 %. The following data were obtained:

T	25·5 mm.	47 mm.	67 mm.	88 mm.
S	24 %	56 %	80 %	88 %

Reference to the figure will show that the curve for blood containing ·04 % lactic acid comes between that of normal blood and that of blood

containing ·08 % lactic acid but on the whole it is nearer to the latter than to the former. These curves are therefore quite analogous to those which would be obtained by adding increasing concentrations of CO, to the blood.

Results obtained in animals.

It is known[4] that asphyxia produces lactic acid in the blood of animals. We therefore determined to ascertain whether complete or partial cutting off of oxygen supply could produce changes in the blood which were large enough to react upon the dissociation curve.

In the case of animals which we wished to breathe air containing a gradually diminishing supply of oxygen we adopted the following method. When anæsthesia with urethane had supervened a ⊣ shaped trachea tube was introduced. To the horizontal limb of the ⊣ was attached a piece of rubber tubing and a clip. To the end opposed to the trachea could be attached a pair of Chauveau's valves. These valves (Fig. 4, *A*) lead from a bottle of about 13 litres capacity. The bottle, which was of the aspirator type, had an orifice near the bottom from which water could be allowed to escape; to this orifice was attached a delivery tube by means of rubber tubing. The delivery tube when turned vertically upwards formed a pressure gauge. The bottle was filled half full of water by means of a funnel *B* which passed through the rubber cork. The glass tube *C* with a tap was passed through the funnel and fixed firmly into it by means of a piece of rubber tubing at *D*. This led from a nitrogen cylinder in the direction of *E* so that by further displacing the water with nitrogen a mixture consisting of air and nitrogen could be obtained containing any quantity down to about 10 % of oxygen.

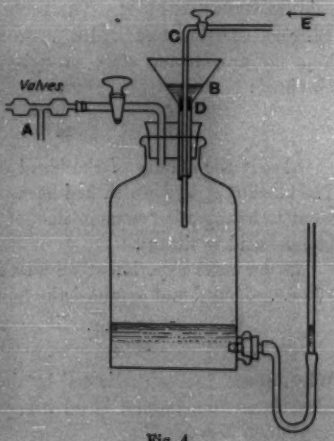

Fig. 4.

When the gaseous mixture was inhaled by the animal from the bottle, it was exhaled into the external air; but it was replaced in the bottle by a stream of nitrogen from the cylinder, so that the level of the water in the gauge was always approximately equal to that in the bottle.

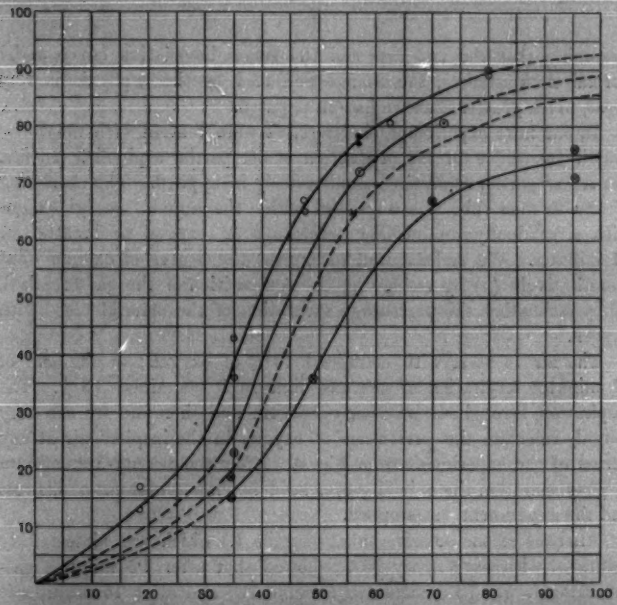

Fig. 5. ○ Defibrinated cats' blood. ⊗ Points from Cat I. after partial occlusion of trachea and 15 minutes breathing of gas of increasing poverty in oxygen. • Cat II. at beginning of exp. ⊙ after 15 minutes gas-respiration. × after 21 minutes ditto.

In the case of the cat I. we first of all restricted the oxygen supply by partially occluding the trachea tube; when the arterial blood became dark, the trachea tube was released and the animal breathed out of the bottle for 15 minutes. The gas to start with contained roughly 10% of oxygen and at the end 4%. Some arterial blood was then withdrawn and its dissociation curve was determined. This curve is shown as the lowermost in Fig. 5.

The uppermost curve is that of a normal cat which was killed by destruction of the brain, bled and the dissociation curve of the blood at once determined. The points on the curve were as follows:

T	18·5 mm.	35 mm.	47·5 mm.	62·5 mm.	80 mm.
S	13 %	36 %	65 %	80·5 %	89 %
	17	48	67		90

Assuming that the two cats had the same dissociation curve under normal circumstances, the change produced by the asphyxia is very great, much greater than we had anticipated.

In our next experiment, therefore, we were content with much less drastic treatment for the production of our partial asphyxia. In fact the term "partial asphyxia" could scarcely be applied. The narrowing of the trachea tube was dispensed with; the animal was given gas with a gradually diminishing percentage of oxygen to breathe for 21 minutes. We endeavoured to express the degree of oxygen want quantitatively (*a*) by estimating the composition of the gas breathed, (*b*) by estimating the percentage saturation of the arterial blood with oxygen.

The air to start with contained 10 % of oxygen; at the end of 21 minutes respiration of the gas mixture it contained 3·6 %. The respiration throughout was fairly uniform and therefore we can very roughly calculate the percentage of oxygen in the inspired air at any period of the experiment from Fig. 6. After the twenty-first minute of gas-respiration the animal commenced to show symptoms of distress and the experiment was stopped.

Analysis of the arterial blood (sample I.) which was almost fully saturated with oxygen, say 95 %, showed that after 1·5 minutes gas-breathing it was 78 % saturated (sample II.) and after 15 minutes it was 52 % saturated (sample III.), the final sample (IV.) unfortunately clotted during the analysis. It was obviously darker than sample III. If the reduction went on at a constant rate it would be about 40 % saturated. Portions of samples I., II. and IV. were shaken with mercury to remove the possibility of their clotting and points on their dissociation curves were determined.

One point only was determined for sample I. (normal blood); the determination was made in duplicate and the point fell on the curve already plotted out for normal cats blood. (Fig. 5. $T = 57$, $S = 77$, 78·5 mean 78.) We felt justified therefore in considering that this curve held good for the particular cat which we were investigating (cat II.).

Three points were obtained from sample III. They are marked thus: ⊙ on Fig. 5. One point was obtained from sample IV., marked by a × on Fig. 6.

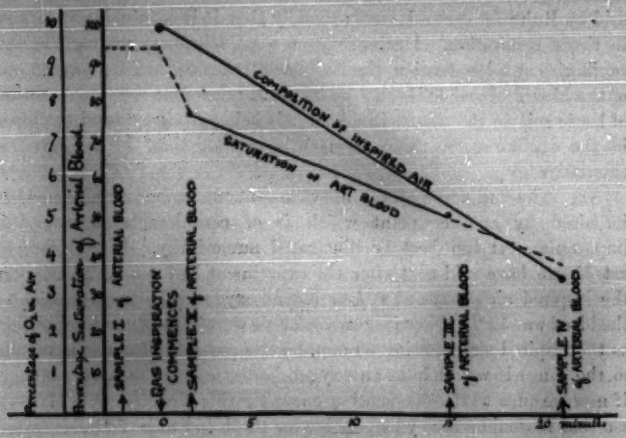

Fig. 6.

The following table gives the data from the curves yielded by three sets of determinations.

	Oxygen in inspired air		Oxygen in arterial blood in animal	Data of dissociation curves T=35	T=57	T=72	
Sample I	21 %	Saturation	95 %	37	78	86	Saturation
Sample III	5·6		52	26	72	82	
Sample IV	3·4		40	—	66	—	

When the results are compared, it appears that as the oxygen want increases the dissociation curve of the blood (at 40 mm. CO_2 tension) becomes progressively more and more depressed, and that this depression is probably due to an increase in the lactic acid in the blood; the curves being quite similar to those obtained by the addition of lactic acid to defibrinated blood.

Furthermore it will be seen by comparing the three points at 56—57 mm. pressure that the rate of lactic acid production is considerably greater in the last six minutes when the arterial blood was less than half saturated, than in the first 15 minutes of the experiment.

Discussion of Results.

(1) The most obvious comment which can be made upon the above experiments is to repeat for lactic acid what has already been said by Bohr for carbonic acid; namely that it is a valuable accessory in tissue respiration. During oxygen want, when oxygen reaches the capillaries at a low tension, the lactic acid tends to turn the oxygen out of the blood. Further it must be borne in mind that the concentration of lactic acid in the tissues in which it is being produced (and therefore in the capillaries of these tissues) is greater than in the general circulation.

(2) The full force of the above argument is, however, somewhat impaired by another point which is of considerable physiological importance. It can best be illustrated numerically. In the case of cat II. we have said that after the experiment had run for 15 minutes the inspired air contained 6 % or less of oxygen and the saturation of the blood was 52 %. Now if the alveolar air were of the same composition as the inspired air (allowing 50 mm. for the pressure of aqueous vapour in the lung) it would have an oxygen pressure of 43 mm. of mercury. If now we turn to the dissociation curve for this blood (sample III.) we see that it would scarcely be possible for the blood to be 52 % saturated when exposed to 43 mm. oxygen tension, and if the oxygen in the inspired air would not produce the observed saturation *a fortiori* that in the alveolar air would be insufficient.

We are therefore face to face with the fact that at 40 mm. CO_2 tension the alveolar air of the cat could not produce the observed saturation of the blood. There appear to be two ways of explaining the facts.

(*a*) The secretion of oxygen by the lung, which is held by Haldane and Douglas[9] to be an active factor during oxygen want.

(*b*) A diminution of the CO_2 tension in proportion to the appearance of lactic acid.

As to (*a*) the whole data upon which this theory is based depends upon determination of the dissociation curves of oxy- and carboxy-hæmoglobin. That of oxy-hæmoglobin is effected by lactic acid; unless the assumption is made that that of CO-hæmoglobin is effected in the same ratio (an assumption which is likely to be correct) the curve of dissociation of hæmoglobin in the presence of O, and CO in the presence of lactic acid would have to be redetermined.

Moreover, underlying the work of Haldane and Lorrain Smith is the assumption that what is not CO-hæmoglobin in the circulating

blood is oxy-hæmoglobin. This assumption is not strictly true. Doubtless at ordinary oxygen pressures the inaccuracy is not material. When, however, oxygen want is produced by oxygen deficiency (as opposed to excessive need) the error may be very considerable. We are therefore in the dark as regards data on which to discuss the secretory theory.

Recent work however has gone in the direction of shewing that the respiratory centre is governed by the total concentration of acid in the blood. We may therefore provisionally accept solution (*b*) as being the true way out of our difficulty.

It must be borne in mind that the value taken for the alveolar tension of CO_2 (40 mm.) is an arbitrary one, and before this discussion could be followed up further experiments would have to be performed.

(3) What has just been said, and indeed everything in the present paper, assumes that the animal is at rest, that the respiratory centre is, as it were, in equilibrium with the blood reaching it; but no doubt the most important result of lactic acid takes place in very different circumstances, under the stress of extreme exercise when the waste products of the body have got altogether beyond the control of the respiration; then indeed the effect on the dissociation curve may be very great. The most beneficial effect to the animal would be when, owing to panting, the oxygen tension in the alveolar air was at its highest and the acid whilst scarcely effecting the saturation of the blood in the lungs would exert a maximal effect in discharging the oxygen from the hæmoglobin in the capillaries.

(4) The effect of lactic acid in cases of drowning &c., where there is in both struggling and stoppage of the respiratory processes, must be very great.

(5) The dissociation of cat's blood at 40 mm. CO_2 tension differs materially from that of the sheep but approximates very closely to that of the dog (Barcroft and Camis).

The expenses of the above research were borne in part by a grant from the Royal Society. The surgical operations were performed by J. Barcroft.

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(1) Bohr, Hasselbach and Krogh. Skand. Archiv f. Physiol. XVI. p. 409.
(2) Barcroft and Camis. This Journal, XXXIX. p. 119. 1909—10.
(3) Barcroft and Roberts. This Journal, XXXIX. p. 429. 1909—10.
(4) Araki. Maly's Jahres-Bericht, XXII. pp. 380—85.
(5) Douglas and Haldane. Proc. of the Royal Society, B, LXXXII. p. 331.

ON THE RECOVERY OF MUSCLE AND NERVE AFTER THE PASSAGE OF A PROPAGATED DISTURBANCE.

By KEITH LUCAS, *Fellow and Lecturer of Trinity College, Cambridge.*

(*From the Physiological Laboratory, Cambridge.*)

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In 1909 I drew attention to the fact that when two adequate stimuli are sent into the sartorius muscle of the frog at an interval little greater than the refractory period, the electric response to the second stimulus appears only after abnormal delay[1]. Since that time I have extended my observations to other tissues. There have also been published papers by Samojloff[2] and by Gotch[3], dealing with similar phenomena.

I. *The electric response of the gastrocnemius muscle of the frog to two stimuli applied to the sciatic nerve.*

Samojloff[4] points out that he had already described in 1908[5] a similar delay in the electric response of the gastrocnemius muscle to a

[1] This *Journal*, XXXIX. p. 331. 1909.
[2] This *Journal*, XL. p. 250. 1910.
[3] *Arch. f.* (*Anat. u.*) *Physiol.* Suppl. p. 1. 1908.
[4] *Zentralbl. f. Physiol.* XXIV. p. 45. 1910.
[4] *Loc. cit.* p. 46.

second stimulus applied to the sciatic nerve. He states[1] however that he took as the time of appearance of the second electric response the point at which the curve of the second response begins actually to turn upwards away from the abscissa. He now observes that in his records, obtained with a string galvanometer, the response produced by the two stimuli diverges from the response to one stimulus alone at a point considerably earlier than the beginning of the actual upward inclination of the second response. On taking the point where the combined curve first diverges from the single curve as the true beginning of the second response he comes to a conclusion opposed to that which he had previously expressed. He finds in fact that the response to the second stimulus takes place on the whole without abnormal delay.

It is essential, if this matter is to be cleared up, that we should have a definite understanding of what is meant by the beginning of the response to the second stimulus. For my own part I have always taken the beginning of the second response to be the point at which the response to the two stimuli first diverges visibly from the response produced by the earlier stimulus acting alone. Or, if I may use Samojloff's terminology, I have measured from the beginning of the deformation of the first curve by the second response. All the measurements which I have published relate to this point. I cannot see that the point previously used by Samojloff, namely that at which the second curve begins actually to be inclined upwards, has any useful significance. The location of that point is determined by the degree of downward inclination of the first curve from which the second is diverging, and by the magnitude of the second response. The use of such a point for measurement of the beginning of the second response is reduced to an absurdity by the fact that one not infrequently obtains a response to two stimuli in which the second response appears in the corrected electrometer curve as a mere decrease in the downward sweep of the first response, and fails to turn upwards (away from the abscissa) at all. In Exp. 1 (Figs. 1 and 2) there are shown two curves corrected by analysis from capillary electrometer records. The first of these (Fig. 1) is the monophasic response of the sartorius muscle of the frog to one stimulus only, the second (Fig. 2) the response to two stimuli at an interval of ·0108 sec. In Fig. 2 there is a well-marked second response, which never leads to an upward inclination of the curve. These curves demonstrate the absurdity of measuring the beginning of a second response from the first upward inclination of the second part of

[1] *Zentralbl. f. Physiol.* XXIV. p. 52. 1910.

the curve. We may assume therefore that Samojloff's more recent conclusion, based on the other method of measurement, represents the more correct interpretation of his curves. In fact the upshot of his work is to show that in the muscle excited from its motor nerve there is no such delay in the electric response to a second stimulus as I have shown to occur in the sartorius excited directly.

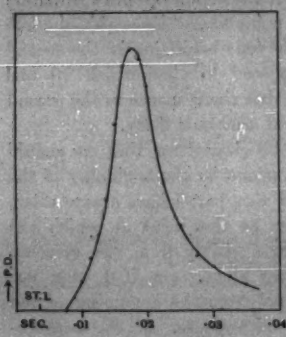

Fig. 1.

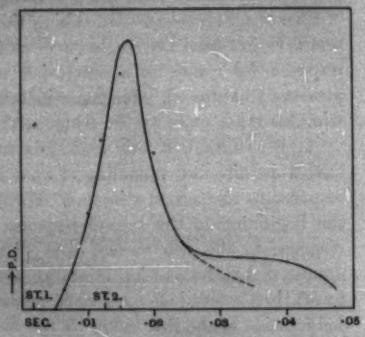

Fig. 2.

The records on which Samojloff's conclusions are based were obtained with a string galvanometer. He speaks of using the string galvanometer in order to "verify his results with a more perfect instrument than the capillary electrometer[1]." In my own experience the string galvanometer though admirably adapted to the recording of such slow changes as occur in cardiac and unstriated muscle, has so much lag that it cannot follow the rapid changes in striated muscle with sufficient exactness to be able to dispense with the necessity of correction. And here it shows itself as vastly inferior to the capillary electrometer; for it is not susceptible of that exact method of correction which gives to the capillary electrometer its peculiar value. On these grounds I thought it worth while to repeat the observations of Samojloff, using as he did the sciatic-gastrocnemius preparation of the frog, and recording the electric responses with the capillary electrometer.

Method. The details of the capillary electrometer, recording apparatus, muscle-chamber, and comparator for the analysis of records, have been described in a previous paper[2]. The gastrocnemius was led

[1] *Loc. cit.* p. 50.

[2] This *Journal*, XXXIX. p. 210. 1909.

off by moist threads attached to its middle and its tendinous end. The cathodes of the coreless induction coils were applied to the nerve at a point about one centimetre distant from the muscle. A series of photographic records was made with two stimuli sent into the nerve at gradually increasing intervals. From time to time during the experiment the response to the first stimulus alone was recorded for comparison with the combined curves. The photographic records were subsequently analysed and plotted with time as abscissæ and P.D. as ordinates. The analysed response to two stimuli was then so superposed upon the response to one stimulus alone that the point of divergence of the combined from the single curve could be measured.

I reproduce here a few of the plotted curves from one of these experiments.

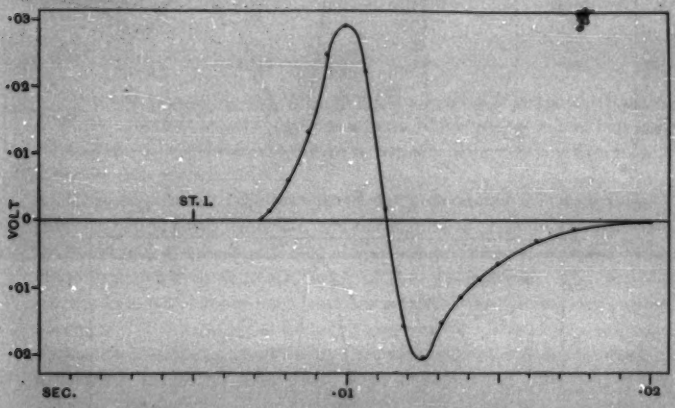

Fig. 3.

Fig. 3 shows the diphasic response of the gastrocnemius to one stimulus sent into the sciatic nerve. When two stimuli were sent in at an interval of ·001 sec. there was no difference in the response, the second stimulus falling within the refractory period. The same occurred when the interval was ·002 sec. An interval of ·003 sec. between the stimuli gave the result plotted in Fig. 4. In this figure the response to the two stimuli is shown as a full line[1], while the response to a single

[1] The first part of the first response is also shown as a broken line in Figs. 4 and 5, because this part of the curve was transferred from Fig. 3, no new analysis being made.

stimulus is continued as a broken line, its course being found by superposing Fig. 3 upon Fig. 4. The point of divergence of the combined from the single curve is marked by a broken line let fall upon the abscissa. This line marks then the beginning of the second response.

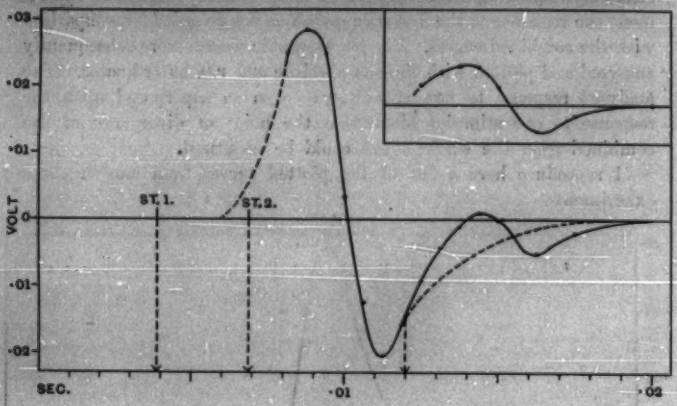

Fig. 4.

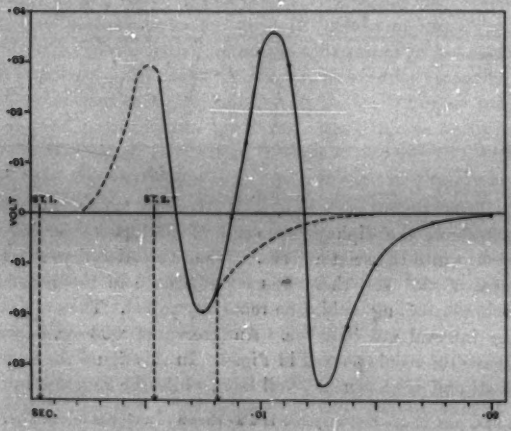

Fig. 5.

The first and second stimuli are marked in a similar way. Fig. 5 shows a similar treatment of the response to two stimuli at an interval of ·005 sec. The second response in this case is very much larger than with the interval ·003 sec. The series was continued to considerably greater intervals between stimuli than those shown in Figs. 4 and 5. For the rest of the curves I shall restrict myself to giving a table of the time of occurrence of the second stimulus and of commencement of the second response for each observation[1].

Exp. 2. Sciatic-gastrocnemius preparation. Frog. Temp. = 17·5° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response	Time second stimulus to second response
B	·001 sec.	No second response	—
C	·002	No second response	—
D	·003	·0081 sec.	·0051 sec.
E	·0037	·0077	·0040
F	·0050	·0077	·0027
G	·0065	·0089	·0023
H	·0147	·0172	·0025

The last column in this table shows the delay of the second response. With the shortest interval between stimuli which gives a second response (Obs. *D*) the delay is practically twice as long as it is when the second stimulus falls considerably later.

This experiment indicates that in the gastrocnemius muscle excited from its motor nerve there is a marked abnormal delay in the response to the second of two stimuli sent in at a short interval of time. But there is an obvious source of error in the observations. The time of commencement of the second response is taken from the first measurable divergence of the combined from the single curve. The electric response always rises with a gradual sweep from zero, so that it does not reach a measurable height instantaneously with its beginning. The smaller the response the longer will be the interval between its actual beginning and the moment when it attains a measurable height. Consequently a small response will not be detected so soon after its actual beginning as will a larger one. It appears from all my observations that the response is smaller the earlier the stimulus by which it is provoked. Is it not possible therefore that the responses to early stimuli appear to be delayed only because they are small and in consequence are detected a considerable time after their actual beginning?

In this connexion the small inset in Fig. 4 should be examined. It shows the second response alone, obtained by subtracting the ordinates

[1] For the analysis of these curves see the Appendix at the end of this paper.

of the single from the combined curve. Measurement shows that the time from commencement to summit of the first phase of this response is about ·0021 sec., whereas that of the first phase of the first response is nearly ·0029 sec. Gotch[1] has demonstrated that the time relations of a small electric response are normally identical with those of a larger one. May not the difference observed here be due to the early part of the second response having escaped detection? Perhaps a more correct rendering of the second response would be obtained by prolonging its time of rise to ·0029 sec.

Considerations such as these make it very probable that there is some error in estimating the time of commencement of the small second response. The question to which we need an answer is whether the error is accountable for the whole of the apparent abnormal delay which my observations show. It seems possible to compensate fully for possible error by making use of the fact that the time relations of large and small responses are alike, and consequently assuming that the second response always begins at a fixed time before the attainment of its maximum P.D.[2]. If we take Exp. 2, and suppose the second responses to begin always at ·0029 sec. before their maxima, as does the large first response, then we get the following values for the times of occurrence of the second response.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response	Time second stimulus to second response
B	·001 sec.	No second response	—
C	·002	No second response	—
D	·003	·0074 sec.	·0044 sec.
E	·0037	·0076	·0039
F	·0050	·0074	·0024
G	·0065	·0091	·0026
H	·0147	·0169	·0022

The last column shows that the general relation of the delay to the time of occurrence of the second stimulus is not materially different from that obtained before. The delay for the earliest second stimulus

[1] This *Journal*, XXVIII. p. 402. 1902.

[2] Gotch has observed (This *Journal*, XL. p. 261, 1910) that in some cases his earlier second responses in nerve show actually a longer interval than the first response between their commencement and their moment of maximum P.D. This, as Gotch points out, is a sign of slowed propagation under the electrode which lies near to the seat of stimulation. But, as he shows, there is no slowed propagation in the region between the electrodes. We need not therefore concern ourselves in these experiments with the possibility that the rise of the first phase of the second response may be longer than normal, since our observations are made at a point far more remote from the seat of stimulation than the position of even the more remote of the electrodes in Gotch's experiments.

is still twice as great as that for the latest. Very similar results are obtained if we assume the time from the commencement of the second response to its point of equipotentiality to be constant in every case.

In order to present in a graphic form the results of the whole of this experiment I have constructed in Fig. 6 a diagram giving the relation between the time of occurrence of the second stimulus and the time of commencement of the second response. In this diagram abscissæ represent time, measured from the occurrence of the first stimulus as zero. Each observation is plotted along a horizontal line, the first stimulus being plotted on the zero line Stim. 1, the second stimulus on the line Stim. 2, and the beginning of the second electric response on the line Resp. 2. The successive observations are plotted at such heights above the abscissa that the second stimuli of all observations fall upon the straight line Stim. 2. If then the second stimulus always produces its response with the same delay, the line Resp. 2 will be a straight line running parallel to Stim. 2 throughout its course. But as a fact the line Resp. 2 runs parallel with Stim. 2 only when the interval between the stimuli is large. When the second stimulus occurs soon after the refractory period, as in observations *D* and *E*, the line Resp. 2 bends away from Stim. 2, showing an increased delay in the appearance of the second response.

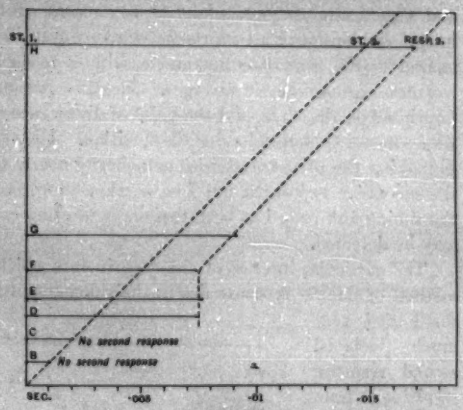

Fig. 6.

I was not completely satisfied with the several experiments which I made by the method described above, because of the difficulty of superposing the combined curves upon the single curves with sufficient accuracy. I thought greater accuracy could perhaps be obtained

by a modification of the method of superposed photographs used by Samojloff[1]. In my experiments the object was to compare the response to the first stimulus acting alone with the response to the two stimuli acting in succession. I have therefore modified Samojloff's procedure, exposing first with the first stimulus alone, and then, without disturbing any adjustment, exposing the same plate with both the first and second stimuli in action. At the second of these exposures the image of the meniscus starts by tracing over its previous course in answer to the first stimulus, and continues to do so until the second response begins, when it diverges. The first response appears strongly imprinted on the plate, and the point of divergence of the fainter second response can be determined with precision. I have found no difficulty in making the plate travel with sufficiently nearly the same velocity in the successive exposures, but I have taken the precaution of inscribing the tuning fork record at both exposures in every case, so that any error may be detected.

The particular interest of the experiments which were made by this method of double exposure lies in their demonstration of an extremely small and very much delayed second response resulting from the application of the second stimulus at the shortest intervals after the first. For stimuli following one another at intervals of ·003 sec. or more these experiments simply reproduced the phenomena already observed by the other method. It was with intervals as short as ·001 to ·002 sec. that the new method detected extremely small responses which might have been overlooked in the earlier experiments. Of course the exact

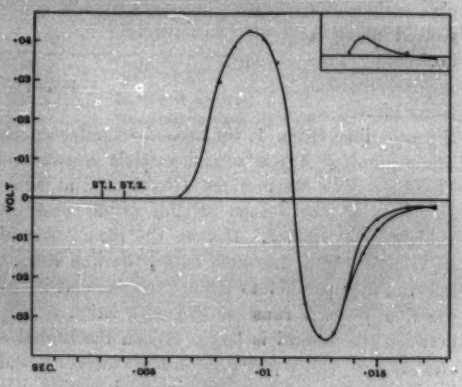

Fig. 7.

[1] *Arch. f. (Anat. u.) Physiol.* Suppl. p. 4. 1908.

point of commencement of these smallest responses was more difficult than ever to determine with precision. They are so small and diverge so gradually from the single response that I have been forced to use the moment of their maximum P.D. as a basis for measuring the time of their beginning. Whatever the method of measurement used there seems to be no escape from the conclusion that they are even more delayed than were the most delayed second responses found in my previous experiments. In Figs. 7 and 8 there are reproduced two of the curves showing these very small second responses. Fig. 9 shows the larger second response produced by a rather later second stimulus. In each case the second response is plotted by itself on a horizontal base-line in the small inset. In the table below (Exp. 3) there are given the data from a whole series of observations made with stimuli at varying intervals.

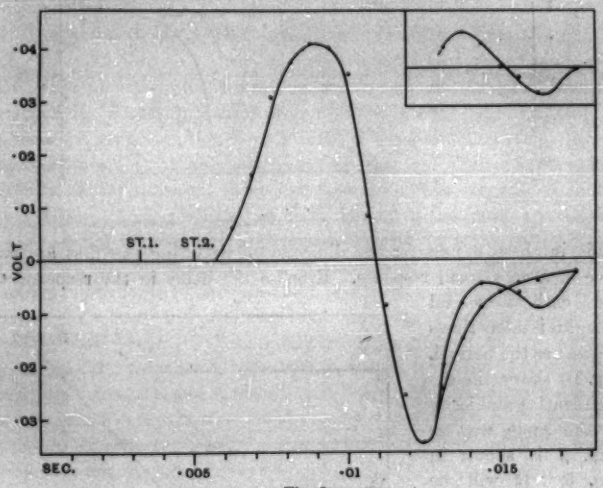

Fig. 8.

Exp. 3. Sciatic-gastrocnemius preparation. Frog. Temp. = 17·5° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response	Time second stimulus to second response
A	·0010 sec.	·0081 sec.	·0071 sec.
B	·0017	·0074	·0057
C	·0025	·0064	·0039
D	·0038	·0066	·0028
E	·0051	·0081	·0030
F	·0072	·0092	·0020

The times of beginning of the second responses are estimated on the assumption that the response begins always at a fixed time (·0031 sec.) before it attains its maximum P.D. Full allowance has therefore been

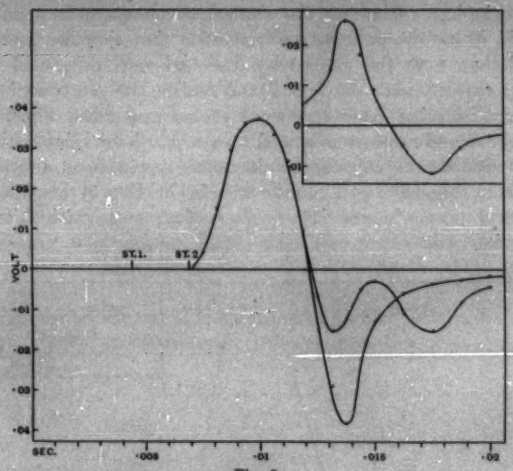

Fig. 9.

made in these figures for the difficulty of detecting the first appearance of a very small response. Even so the delay in the response to the earliest second stimulus is many times as great as the normal. Fig. 10 shows the experiment graphically in the same way as Exp. 2 is shown in Fig. 6. It will be seen that the line Resp. 2 is by no means parallel to Stim. 2 throughout its course. It is nearly parallel for the longer intervals between stimuli, but

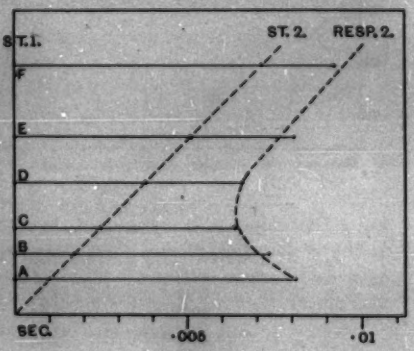

Fig. 10.

for the shorter intervals it diverges very widely, indicating a delay increasing rapidly with the earlier incidence of the second stimulus.

This experiment confirms the result obtained in Exp. 2. The analysed electrometer records show that in the gastrocnemius muscle excited from the sciatic nerve the response to a second stimulus falling soon after the end of the refractory period exhibits a delay which may be many times as great as the normal delay. This conclusion is in direct opposition to that expressed by Samojloff. To account for the difference between the results obtained by Samojloff and myself I can only suggest that possibly the uncorrected string-galvanometer records do not give so true a picture of the changes of P.D. as the corrected electrometer records.

II. *The electric response of the sartorius muscle to two stimuli applied directly to the muscle-fibres.*

The observations on the response of muscle to a second stimulus which I published in a previous paper[1] were based on estimations of the time at which the second response first reaches a measurable magnitude. In the preceding section of the present paper I have given reasons for supposing that such a method of measurement may involve serious error. It is probable therefore that my former observations overstated the abnormal delay which occurs in the response of muscle to a second stimulus falling just after the end of the refractory period.

It should be possible to correct such error by treating my former curves in the manner described in the preceding section, namely, by taking as the basis of measurement the point of maximum P.D. of the second responses, and assuming that they began at a constant time before that point. Unfortunately in the series of curves which I published the response to the earliest second stimulus (Fig. 3, p. 334) is a small curve of almost flat summit, which does not allow more than a very rough determination of the point of maximum P.D. As nearly as I can measure the curves the times of commencement of the second responses are the following.

Curve	Time first stimulus to second stimulus	Time first stimulus to beginning of second response on assumption that response begins ·006 sec. before its maximum P.D.	Time second stimulus to beginning of second response
3	·007 sec.	[·014]	[·007]
4	·0095	·0155	·006
5	·0120	·017	·005
6	·0172	·021	·0088

[1] This *Journal*, XXXIX. p. 331. 1909.

These figures indicate that there is a large increase of the delay (last column) as the incidence of the second stimulus becomes earlier. The measurement of Curve 3 is however too uncertain. I decided therefore to make fresh experiments on the sartorius muscle, giving particular care to the precise determination of the point of maximum P.D. in the second response.

The method of observation was identical with that used in the earlier experiments[1]. The muscle was kept at a constant temperature in the special chamber described, was excited at its pelvic nerve-free end, and led off by moist threads attached to its middle and tibial end. The results of two experiments are given below. In each case the measurement actually made was from the incidence of the first stimulus to the summit of the second response[2]. From this value the interval between the first stimulus and the beginning of the second response was obtained by subtracting the observed interval between the beginning of a normal single response and its moment of maximum P.D. The interval subtracted in Exp. 4 was ·0050 sec. In Exp. 5, which was made at a temperature 10° C. higher, the interval subtracted was ·0035 sec.

Exp. 4. Sartorius of frog. Direct stimulation. Temp. = 8° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to beginning of second response	Time second stimulus to beginning of second response
A	·0028 sec.	No second response	—
B	·0052	·0187 sec.	·0135 sec.
C	·0075	·0183	·0108
D	·0100	·0185	·0085
E	·0125	·0198	·0073

Exp. 5. Sartorius of frog. Direct stimulation. Temp. = 18° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to beginning of second response	Time second stimulus to beginning of second response
A	·0018 sec.	·0107 sec.	·0089 sec.
B	·0030	·0110	·0080
C	·0039	·0110	·0071
D	·0046	·0105	·0059
E	·0073	·0115	·0042

[1] This *Journal*, XXXIX. p. 207. 1909.

[2] Such a correction for possible error seems hardly necessary in this case, since the second responses were all fairly large, and consequently the results agree fairly closely whether the time of beginning of the second response is obtained by this method or by direct measurement of the first measurable divergence of the curves. For example the direct method gives for Exp. 4 *B* and *C* the intervals ·0170 sec. and ·0175 sec., while the indirect method gives ·0187 sec. and ·0183 sec. In Exp. 5 the times ·0107 and ·0110 found by the indirect method become ·0120 and ·0122 by the direct method. With either method the general relations remain essentially the same.

The change of temperature between the two experiments makes a large difference in the refractory period of the muscle. In Exp. 4 the second stimulus at ·0028 sec. is not effective; in Exp. 5 at 18° C. the second stimulus at ·0018 sec. is effective. But at both temperatures the phenomenon of the increased delay with earlier incidence of the second stimulus is essentially the same. As the last columns of the tables show, the delay is practically doubled within the range of each experiment. In Fig. 11 the relation between the time of occurrence of the second stimulus and the time of commencement of the second response is shown for Exps. 4 and 5.

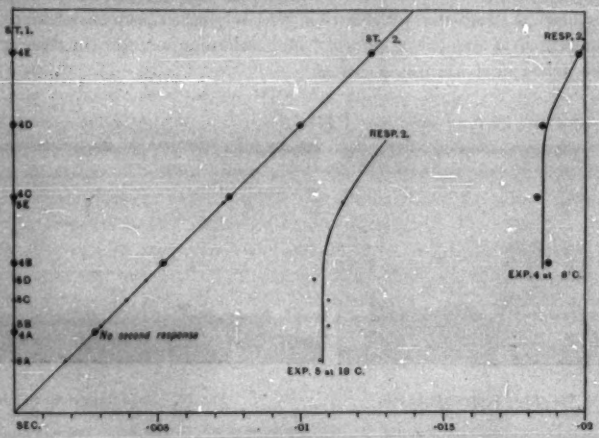

Fig. 11.

These experiments confirm the conclusion which I reached in my previous paper, that in the sartorius muscle excited directly the response to a second stimulus falling soon after the end of the refractory period occurs after abnormally long delay.

III. *The electric response of cardiac muscle to two stimuli.*

The problem of the delay in the response to a second stimulus is far more easily attacked in the case of cardiac than of skeletal muscle. In the latter the earliest possible second response occurs at a time when the first response has not yet subsided, so that we are faced with the

difficulty of analysing the complex response to two stimuli into its components and determining the exact point at which the second response begins to diverge from the first. In cardiac muscle the earliest second response does not occur until the first has completely subsided. Both first and second responses spring therefore from a horizontal base-line, and can be compared on equal terms. Also in cardiac muscle the second response is not appreciably altered in size when it occurs soon after a previous one; by this fact a source of error is removed which was of serious magnitude in the measurements made on skeletal muscle. These circumstances, coupled with the slow time rate of the electric changes in cardiac muscle, render unnecessary the correction of the capillary electrometer curves when the only object is to determine the interval between the occurrence of a stimulus and the beginning of the consequent electric response.

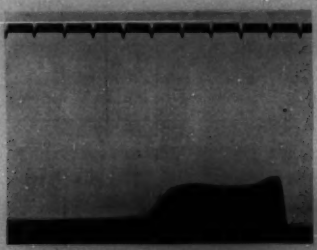

Fig. 12. Read from right to left.

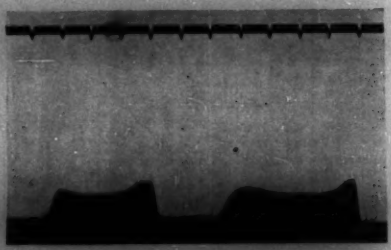

Fig. 13. Read from right to left.

My experiments were made on the ventricular muscle of the frog. Two methods were used. The first of these consisted in stopping the heart by the Stannius ligature, setting it up in the muscle chamber with electrodes applied to one uninjured and one injured point on the ventricle and recording with the capillary electrometer the monophasic responses to two single-induction shocks sent in at varying intervals. The cathodes of the induction coils were applied either to the auricle or to a point between the base of the ventricle and the proximal leading-off electrode. The injury was always under the leading-off electrode most distant from the base of the ventricle.

This method was sufficient to determine without any doubt that the delay of the electric response to a second stimulus occurring soon after

the end of the refractory period is many times as great as the normal delay. Exp. 6 (Figs. 12 and 13) illustrates this point. In these records the time marker shows fifths of seconds, and the moment of stimulation is marked by an escape of the current from the induction coil. In Fig. 12 the second stimulus occurs just before the end of the refractory period, and there is no second response. In Fig. 13 the second stimulus is just after the end of the refractory period, and provokes a second response after a delay many times as long as the delay between the first stimulus and first response.

The second method was to use the intact beating heart, and to send in stimuli at varying intervals after the electric response accompanying a spontaneous beat. This method was adopted because the Stannius preparation is apt to begin beating when stimuli have been sent into it frequently. The second method is therefore better adapted for obtaining a long series of observations with stimuli sent in at widely different intervals after a response. In this way I was able to map out a curve relating the delay of the second response to the time of occurrence of the stimulus. The two tables given below show the results obtained. For convenience of reference the observations are given in a definite order, those with the earliest stimuli coming first. In the actual work the observations were made at random in no such order, so that there cannot be any question of the longer delay observed with some stimuli being due to a progressive change in the tissue. The lettering of the observations shows the actual order in which they were made, *A* being the first in each experiment.

Exp. 7. Ventricle of frog. Stimulus near base of ventricle. Temp. = 15·5° C.

Obs.	Time from beginning of first response to stimulus	Time from beginning of first response to beginning of second response	Time from stimulus to second response
I	·44 sec.	No second response	—
M	·50	1·44 sec.	·94 sec.
L	·62	1·45	·83
F	·68	1·48	·80
K	·73	1·49	·76
G	·73	1·50	·77
E	·86	1·57	·71
D	·87	1·59	·72
B	1·10	1·73	·63
A	1·13	1·75	·62
H	1·20	1·82	·62
C	1·24	(1·60)	(·36)
J	1·46	2·02	·56

In these observations the delay (last column) increases with the earlier incidence of the stimulus until it is nearly doubled. The two stimuli which fall nearest to the end of the refractory period give responses beginning at nearly the same interval after the beginning of the first response (1·44 sec. and 1·45 sec. in the third column). There is one divergent observation, namely obs. *C*, which gives a delay quite out of agreement with the rest; for this I have no explanation to offer. During this experiment the heart was beating at a rate which gave 2·88 sec. between the beginnings of two spontaneous electric responses.

Exp. 8. Ventricle of frog. Stimulus in auricle. Temp. = 15° C.

Obs.	Time from beginning of first response to stimulus	Time from beginning of first response to beginning of second response	Time from stimulus to second response
M	·46 sec.	No second response	—
N	·58	1·49 sec.	·91 sec.
L	·70	1·48	·78
I	·91	1·59	·68
J	·92	1·59	·67
K	·98	1·64	·66
Q	1·00	1·63	·63
F	1·07	1·68	·61
R	1·11	1·73	·62
E	1·23	1·83	·60
C	1·27	1·91	·64
G	1·29	1·87	·58
D	1·41	2·02	·61
B	1·41	2·02	·61
A	1·45	2·07	·62
P	1·46	2·03	·57
H	1·58	2·14	·56
O	1·67	2·22	·55

Exp. 8 reproduces very closely the facts already seen in Exp. 7. In the latter the delay of the second response ranges from ·56 sec. to ·94 sec.; in the former it ranges from ·55 sec. to ·91 sec. The observations from Exp. 8 are shown graphically in Fig. 14. This diagram is constructed in the same way as Figs. 6, 10 and 11 to show the relation between the time of incidence of the stimulus and the delay of the consequent response. The only difference is that in Fig. 14 the zero line from which times are counted is the beginning of the first response, and not the first stimulus, since the first response was always a spontaneous one. The figure shows that when the stimulus falls between 1·0 and 1·5 sec. after the beginning of the first response the

delay of the second response is practically constant at ·6 sec. With earlier stimuli the delay becomes longer. For stimuli between ·5 sec. and ·7 sec. after the beginning of the first response the delay increases so rapidly that the second response occurs at an almost constant time. Earlier stimuli than these fall within the refractory period and produce no second response. During this experiment the spontaneous responses were following one another at an interval of 2·4 sec.

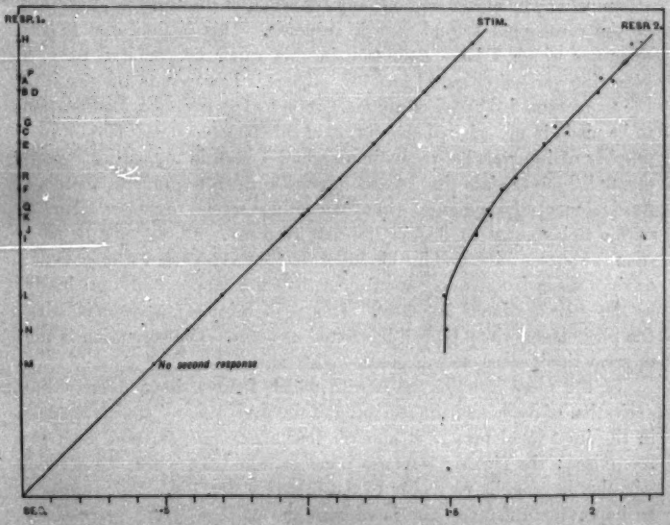

Fig. 14.

We have in these experiments on cardiac muscle a proof of the existence of the increased delay of a second response based on observations free from any difficulty of measurement and demanding no minute analysis of the photographic curves. The agreement of these results with those of the more difficult observations on the rapid tissues is a valuable confirmation. It leads to the conclusion that an abnormally long delay in the response to stimuli falling just after the refractory period is a phenomenon common to different excitable tissues.

IV. *Discussion of the phenomenon of the delayed second response.*

The observations which I have made on skeletal muscle excited directly and on cardiac muscle show in common not only a delay of the second response increasing with the earlier incidence of the second stimulus, but also the phenomenon which I previously described as the "irresponsive period[1]." In other words there is a range of time, succeeding the end of the refractory period, within which the second stimulus may be moved without any alteration of the time of occurrence of the consequent second electric response. This is indicated in the diagrams which I have published in this paper by the line Resp. 2 becoming vertical (Figs. 11 and 14). There is however, as the result of the more extended range of my more recent experiments, a modification to be made in my original conception of this phenomenon. The limited number of intervals between stimuli which I used in my earlier experiments led me to state that second responses which began later than the fixed moment of commencement of the earliest second responses occurred with a delay equal to that of the first response. It can now be seen from the curves published in this paper that such responses still continue to show a considerable increased delay, which disappears only gradually as the second stimuli fall later. This is in complete agreement with the phenomena which Gotch has found in nerve. The correction is one of great significance, as will presently be shown.

In the more complex cases with which I have dealt, namely the excitation of nerve and the recording of the consequent electric response in the innervated muscle, it appears that a new phenomenon must be recognised; the electric response becomes still later when the second stimulus is brought very near to the first (see Fig. 10). This suggests an important difference associated with the passage of the propagated disturbance through successive tissues having unlike time relations. It may be a profitable subject of investigation when the more simpler

[1] This name is not satisfactory, because it does not indicate the essential feature of the phenomenon, that it is a question of the delay of responses to a fixed moment, not of their complete failure as in the refractory period. For this reason I have not used the expression in the present paper. At the same time it is obviously inconvenient to refer constantly to "the period to the end of which the responses to the earlier second stimuli are delayed." Gotch (This *Journal*, XL. p. 272, 1910) uses the expression "period of modification" for the whole time during which the second response is delayed more than the first; but this includes a time longer than the period to which I am referring. It will perhaps be better to adopt no special terms for these phenomena until further experiment has thrown more light on their relations.

cases, where only one kind of tissue is concerned, are better understood. At present I shall confine my discussion to the single tissues.

In addition to those observations on the sartorius muscle and the ventricle which I have described there is the long series of measurements made by Gotch[1] on the sciatic nerve of the frog. He sums his observations up in the following words: "There is some evidence that at low temperatures (3° C.) second nerve responses commence at the same delayed moment, although the second stimulus is varied as regards its moment of application. This corresponds with the results obtained by Keith Lucas in the sartorius muscle when directly excited by two stimuli at 12° C. Since however at temperatures above 3° C. the moment of commencement of the second response of nerve varies with the time of the application of the second stimulus, the conclusions arrived at by Keith Lucas as regards a fixed terminal limit of an 'irresponsive period' are not applicable to nerve." Now my recent observations on cardiac and skeletal muscle show that the occurrence of the second responses at the same delayed moment is only to be expected when the second stimuli fall quite soon after the end of the refractory period. And (as may be seen from Fig. 11) the greatest interval at which the second stimulus may follow the first while still producing its response at a constant moment becomes smaller as the temperature is raised. We ought therefore to enquire, before postulating a real difference between muscle and nerve in this matter, whether Gotch's observations have always explored the region within which the occurrence of second responses at the same delayed moment is to be expected.

Gotch's experiments at each temperature consist in most cases of several groups of two or three observations derived from different preparations. It is clear I think that we shall not be justified in considering together measurements made on different preparations; we cannot rely on the identity of two nerves within the narrow limits of time measurement demanded by these experiments. The observations made on each preparation must stand alone.

Starting with the lower temperatures we have at 3° C. five observations all made on the same preparation.

Group A. Temp. = 3° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response
967	·0110 sec.	·0150 sec.
966	·0120	·0150
965	·0130	·0155
964	·0140	·0160
963	·0150	·0165

[1] This *Journal*, XL. p. 253. 1910.

This is the case to which Gotch refers as agreeing with my observations on muscle. The second response occurs at ·0150 sec. with stimuli ·0110 and ·0120 sec. apart. It should be noticed that Gotch gives the refractory period of this preparation as ·0075 sec., so that the constant time of the second response may have occurred with second stimuli over the whole range from ·0075 to ·0120 sec.

At 4° C. there are observations from three preparations.

Group B. Temp. = 4° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response
1071	·0100 sec.	·0120 sec.
1072	·0125	·0135
997	·0080	·0135
996	·0100	·0135
969	·0090	·0135
970	·0100	·0135

The refractory period is ·007 sec. In observations 1071 and 1072 the second stimuli follow the first at too great an interval to show second responses commencing at the same delayed moment. Both the other preparations, with stimuli nearer to the refractory period, do give second responses occurring at a fixed time.

At 4½° C. the results are derived from four preparations as follows.

Group C. Temp. = 4½° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response
1064	·0100 sec.	·0125 sec.
1063	·0120	·0135
1014	·0070	·0110
1015	·0080	·0110
1016	·0100	·0125
1125	·0080	·0120
1137	·0080	·0110

The single observations are of no use for our purpose, except in showing that measurements made on different tissues with the same interval between stimuli do not agree. The observations 1064 and 1063 are made with stimuli too far apart. The group of three observations made with second stimuli closer to the end of the refractory period (given as ·007 sec.) brings out the fixed time of occurrence of the second responses again quite definitely; stimuli at ·0070 sec. and ·0080 sec. both have their responses at ·0110 sec.

At 5° C. the observations are made with different distances between electrodes and point of stimulation, and no indication is given of the preparations used, so that the results cannot be used here. At 8° C. there are observations from three preparations.

Group E. Temp. = 8° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response
1001	·0045 sec.	·0075 sec.
1000	·0065	·0088
999	·0080	·0100
1022	·0050	·0080
1021	·0060	·0080
1011	·0040	·0070

The observations 1001 to 999 do not show the occurrence of second responses at a fixed time; the observations 1021 and 1022 do show it. Neither of these sets is carried right down to the end of the refractory period, which is given as ·0035 sec.

Finally, at 12° C. there is a group of three observations all made on the same preparation and not showing second responses at the same delayed moment though carried to within ·0007 sec. of the end of the refractory period.

Group F. Temp. = 12° C.

Time first stimulus to second stimulus	Time first stimulus to second response
·0027 sec.	·0040 sec.
·0035	·0046
·0045	·0055

We find, then, on considering together only observations made on the same tissue, the occurrence of responses at the same delayed moment at 3°, at 4° and at 4·5° C. whenever the stimuli are brought near enough to the end of the refractory period. There are observations showing that the range of time after the end of the refractory period within which the second stimulus may be moved, without movement of the second response, is as great as ·0045 sec. at 3° C., ·003 sec. at 4° C. and ·001 sec. at 4·5° C. It is also demonstrated that the range is not greater than ·0055 sec. at 3° C., ·0055 sec. at 4° C. and ·003 sec. at 4·5° C. Clearly the range is becoming rapidly shorter as the temperature rises. At 8° C. one preparation shows the phenomenon occurring with stimuli up to ·0025 sec. after the end of the refractory period; the other preparation shows that at ·003 sec. after the end of the refractory period, the range

within which the phenomenon is observed has not been reached, but for want of earlier stimuli gives no indication whether the stimulus at ·001 sec. after the refractory period does or does not fall within that range. At 12° C. it is shown that a stimulus ·0015 sec. after the end of the refractory period does not fall within the required range, there is no indication whether the stimulus ·0007 sec. after the end of the refractory period does or does not.

The conclusion which I would draw from these observations is that in nerve the occurrence of a response at the same delayed moment to stimuli sent in at varying short intervals after the end of the refractory period has been demonstrated at temperatures from 3° C. to 8° C.; that the range of time after the end of the refractory period within which stimuli must fall if their responses are to occur at the same delayed moment becomes rapidly shorter with rise of temperature; that at 12° C. there is no evidence whether such a range exists at all, but if it does it is certainly shorter than ·0015 sec. In these facts I cannot find reason for supposing the phenomena in nerve to differ in any way, save only in absolute time values, from those which I have described as occurring in skeletal and cardiac muscle.

As to the meaning of the delay of the second response to a fixed moment, it is obviously very tempting to accept Gotch's illuminating suggestion that the second response is delayed because it is propagated along the muscle or nerve more slowly than the first. This not only fits in with the observation of Engelmann[1] that in the ureter a second wave can be seen to progress at a rate decreasing with the time interval since the passage of a previous wave, but it offers a simple account of the fixed time of occurrence of responses to stimuli sent in at different times. If the first propagated disturbance sets up in the tissue some modification which persists at each point for a definite time after the disturbance has passed and is of such a nature as to slow the propagation of a subsequent disturbance, then it is obvious that the second disturbance will be slowed in its propagation if it finds the modification still persisting at any part of the tissue which it enters; but as soon as, in consequence of such slowing, it comes to lag so far behind the earlier disturbance as to find each part of the tissue on which it enters just recovered from the modification, then it will no longer be slowed, but will follow the former disturbance at an equal pace. The result of such a mechanism would be that the second disturbance, provided only that it set out before the modification due to the first had

[1] *Arch. f. d. ges. Physiol.* II. p. 268. 1869.

subsided, would come to follow the first at a fixed interval equal to the duration of the modification which the first disturbance had set up.

If this were all, the matter would be simple. But in this scheme no account is given of the fact clearly established by the experiments of Gotch as well as by my own, that second responses which, owing to their stimuli falling too late, begin later than the fixed limit are nevertheless slightly delayed as compared with responses occurring in a tissue not recently excited. Such a result could not arise in the simple way which I have suggested above. It might however result from some local modification of the tissue at the seat of the first stimulus lasting slightly longer than the supposed modification due to the propagated disturbance. In this way Gotch's suggestion that the delay is partly due to a local factor resulting from the passage of the exciting current, and partly the consequence of a modification set up by the propagated disturbance, might account for all the observed facts. This tempting hypothesis is at once ruled out by the occurrence of an increased delay in responses falling later than the earliest limit in cardiac muscle. The first response in my experiments on cardiac muscle was a spontaneous one, so that there cannot have been any local modification set up by an exciting current. All the phenomena of delay observed in the experiments on cardiac muscle must have their origin in modification of the tissue by a previous propagated disturbance only.

This preliminary survey of the causation of the increased delay forced me to recognise that the first necessary step was a close investigation of the factors concerned, and particularly a determination of the relative importance of such modifications as may be set up by the stimulus itself and by the consequent propagated disturbance. The following section of this paper is an account of the experiments which I have made upon this question.

V. *Investigation of the factors concerned in the increased delay of the second response.*

We have here two separate problems to solve. There is the question which of the changes produced in a tissue by the first stimulus is the condition delaying the second response. Is it the local effect of the exciting current at the seat of excitation, or is it the consequent propagated disturbance? And there is the problem how the delay is

effected. Is it by a slower development of the second local process of excitation, or by a slower propagation of the second disturbance?

In his paper on the delay of the electrical response of nerve to a second stimulus Gotch has dealt with the first of these problems. He reaches the conclusion that there are two factors concerned, namely "stimulation fatigue," or the local impairment of the tissue by the electric current at the actual seat of stimulation, and "functional fatigue," or the impairment of the tissue over the region which has previously been traversed by a propagated disturbance. Gotch brings quite definite evidence of the reality of the second of these factors, when he shows that local cooling or warming of portions of a nerve may alter the amount of the increased delay, although the locality exposed to the alteration of temperature is not the seat of the exciting stimulus. This observation leaves no room for doubt that the delay is in part at least caused by some change brought about by the actual passage of the propagated disturbance alone the nerve.

The evidence as to the importance of the other factor, namely, stimulation fatigue, does not appear to me to be equally convincing. The experiments which are held to indicate that stimulation fatigue is partly responsible for the delay in the response to a second stimulus are of two kinds. First, it is shown that if an interrupted current is applied to a nerve for five minutes, and the response to a single stimulus is taken a few seconds after the cessation of the interrupted current, that response occurs after abnormal delay[1]. If however the stimuli used for provoking the test responses are not applied at the seat of prolonged stimulation, then the increased delay is comparatively slight[2].

This experiment affords clear evidence that stimulation fatigue can be induced by prolonged stimulation; but it does not appear to touch the problem with which we are now concerned. We have to deal with a modification produced by one single stimulus and lasting in the case of nerve for a time of the order of a hundredth of a second. It may be that the stimulation fatigue set up by a single stimulus is of negligible magnitude, and it may be that the functional fatigue lasting for a hundredth of a second after a single stimulus is of such considerable magnitude as to outweigh entirely the stimulation fatigue during that time. The fact that at an interval of several seconds after a stimulation of several minutes the stimulation fatigue is proved to be the more important tells us nothing of the relative importance of the two fatigues at the time with which we are concerned; and it is just their relative

[1] *Loc. cit.* p. 263, Fig. 6. [2] *Loc. cit.* p. 265, Fig. 8.

importance which we need to know. The increased delay which we observe must be determined by that fatigue which is most important during the actual time of the delay. In fact the question is whether in observing the increased delay we are obtaining a measure of the duration of stimulation fatigue or of functional fatigue.

The second line of evidence adduced to show the importance of stimulation fatigue appears to be of far greater value. Gotch states "A further confirmation of this is shown by some of my records with an inadequate first stimulus, these show increased delay in the response to the second and only adequate exciting agent[1]." If a single stimulus so weak as to set up no propagated disturbance still causes stimulation fatigue enough to effect a considerable increased delay, a fortiori the stimulation fatigue of a stimulus of adequate strength will be of real importance. Unfortunately Gotch has given us no further details of this interesting line of experiment.

In view of these considerations I was unable to regard the relative importance of "stimulation fatigue" and "functional fatigue" in causing the increased delay as being sufficiently closely determined. I have attempted to devise experiments by which some further light might be thrown upon the problem in the case at least of the response of muscle to a second stimulus.

My object was to devise an experiment by which it should be possible to differentiate directly between the delay caused by the passage of the exciting current as such, and the delay caused by the consequent occurrence of a propagated disturbance. For this purpose it was necessary to compare the effect of an exciting current of adequate strength, which nevertheless failed to set up any propagated disturbance, with that of the same current when it did set up a propagated disturbance. This result was obtained by the use of a stimulus falling within the refractory period. The experiments were made in the following way. In the first observation the plate was exposed to the response of a sartorius excited by a single stimulus, which I will call *A*. At the second exposure of the same plate the stimulus *A* was followed by a stimulus *C*, which fell shortly after the end of the refractory period. This observation gave then an ordinary first response with a delayed and modified second response superposed. The second observation consisted in a first exposure with stimulus *A* as above, and a second exposure with the stimuli *A* and *C* and also a stimulus *B* so timed as to fall within the refractory period of *A*. The third observation was made

[1] *Loc. cit*, p. 266.

with stimulus B alone, to show that it was adequate to produce a full response when acting alone. The fourth observation was made with A alone at the first exposure, and A followed by B at the second exposure, to show that B did actually fall in the refractory period of A. If now the modification of the response to stimulus C observed in the first observation were due in part or wholly to the fact that A had a local effect on the muscle apart from the propagated disturbance which it set up, then in observation 2 there ought to be greater modification of the response to C, since not only was C preceded by two currents instead of one, but the second of these, namely B, occurred at a shorter interval

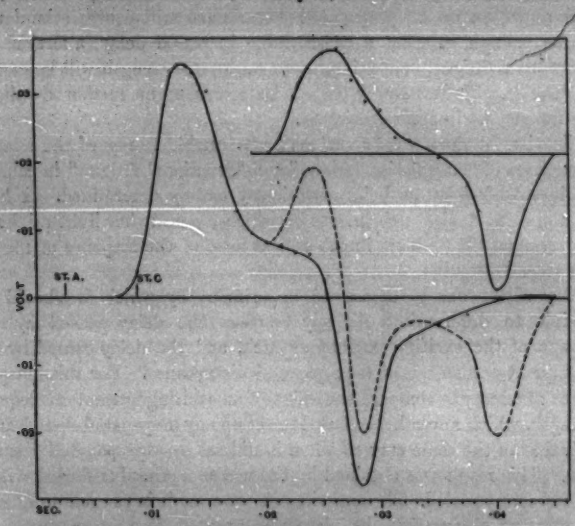

Fig. 15.

before C than did A in the first observation. If, on the other hand, the modification of the response to C were due entirely to the fact that A set up a propagated disturbance, and not at all to any local effect which it had on the muscle, then the response to C ought to be identical in the first observation and the second, that is whether the current B was sent in or not, since B did not set up any propagated disturbance.

The result of this experiment is that the response to stimulus C is not materially altered by the interposition of the stimulus B between

A and *C*, provided that *B* falls in the refractory period of *A*. I give in Figs. 15 and 16 the analysed curves from two such observations (Exp. 9). I have chosen this experiment out of all that I made because it is the only one in which even a very slight difference between the second responses in the two observations could be made out on superposing the photographic curves. The response to *A* is shown as a full line, that to *C* diverges as a broken line. The complicated path which the latter follows proves, on subtraction of the ordinates as shown in the curves plotted on a higher level, to be nothing but a simple diphasic curve. It will be observed in Fig. 15 that the stimulus *C* has been so timed as to produce a response with considerable increased delay. The summit of the first response occurs about ·011 sec. after stimulus *A*, that of the

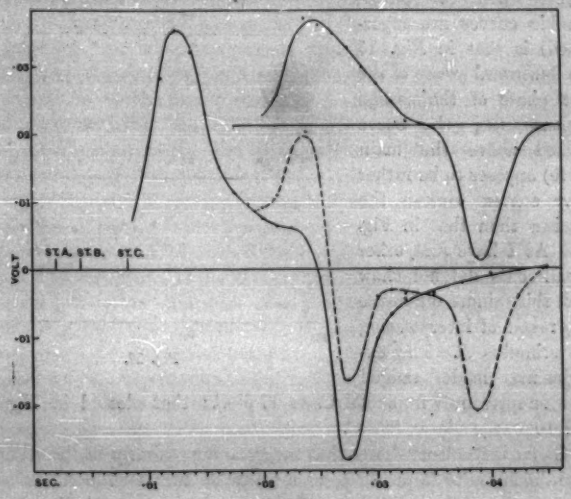

Fig. 16.

second response about ·017 sec. after stimulus *C*. If we suppose the time from beginning to summit of a response to be about ·006 sec. (which appears from Fig. 15 to be the approximate value) then the delay of the response to *A* is approximately ·005 sec.; that of the response to *C* ·011 sec. Clearly the stimulus *C* falls well within the range of time which gives a delayed and modified response. Within this range, as

may be seen from other curves published in this paper, a shifting of the second stimulus may alter the time of occurrence of the second response very little, but will alter very largely the size (degree of modification) of the second response. If then the interpolated stimulus *B* has any effect we should expect it to produce rather an increased modification than an altered delay of the second response. As a fact it appears to produce neither. The second response in Fig. 16 agrees closely with that in Fig. 15 both in size and in time relations. The one slight difference between the curves (which I mentioned above as being visible when the photographic curves are superposed) is that in Fig. 15 the downward sweep of the first phase of the second response (see the curve plotted above the main curve) appears to be rather more convex towards the abscissa than that in Fig. 16. As I have said, other experiments did not show even this minute difference as a result of interpolating the stimulus *B*. The experiments made ranged over temperatures from 8·5° C. to 17·5° C.; that plotted in Figs. 15 and 16 was made at 8·5° C.

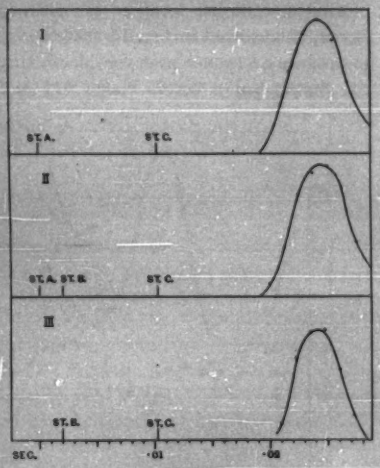

Fig. 17.

So far it has been shown that the greater proximity of the stimulus *B* to the stimulus *C* produces no measurable increased modification in the response to *C*, provided that *B* sets up no propagated disturbance. It remains, in order to complete the evidence, to show that if a stimulus which does set up a propagated disturbance is brought as near to *C* as *B* is, then the modification of the response to *C* will be actually increased by a measurable amount. This was tested in Exp. 10, the results of which are plotted in Fig. 17. This figure shows only the second responses in each case, obtained by subtracting the ordinates of the single response from those of the combined curve. The procedure of

this experiment was as follows. In the first observation (marked I in the figure) the first exposure was with stimulus *A*, the second with *A* followed by *C* at an interval of ·0102 sec. In the second observation the first exposure was with stimulus *A*, the second with *A* followed by *B* at ·0020 sec. and *C* at ·0102 sec. In this case *B* fell within the refractory period of *A*, and the delayed response to *C* was practically identical with that in observation I. A third observation was then made, the first exposure with *B* alone, the second with *B* followed by *C* at the same interval as before, namely, ·0082 sec. (*i.e.* ·0102 – ·0020 sec.) This observation showed the effect of bringing the stimulus which set up a propagated disturbance to the same distance from *C* as was the stimulus *B* in the second observation. The result was a marked increase in the modification of the response to *C*. The E.M.F. reached in the first phase of the response to *C* was reduced in the ratio 46 : 39[1].

I conclude from these experiments that in the sartorius muscle the factor of importance in causing the modification of a second response is the setting up by the first stimulus of a propagated disturbance, and not in any measurable degree the local effect of the actual passage of the electric current at the seat of stimulation. In other words these experiments show that the effect of the stimulation fatigue produced by a single stimulus of maximal strength is not measurable as increased modification of the subsequent response.

I have attempted to control this result by an entirely different method of experiment. If the delay of the response to a second stimulus is due entirely to the occurrence of a previous propagated disturbance, and not to a local effect at the seat of previous stimulation, then the delay ought to be the same whether the second stimulus is sent in at the same point as the first stimulus, or at any other part of the tissue which is passed over by the propagated disturbance. My experiments upon this point have been made with the sciatic-gastrocnemius preparation of the frog excited from its motor nerve. The sartorius muscle, owing to its complex composition, does not lend itself to stimulation with like stimuli at two different points. The difficulty of this line of experiment is that the interval between the beginning of the first propagated disturbance at the seat of the second stimulus and the

[1] If the modification of the second response in Exp. 9 had been due to the "stimulation fatigue" set up by the previous stimulus, we should have expected the interpolated stimulus *B* to reduce the second response by an amount considerably greater than this change of 46 : 39 observed in Exp. 10. For in Exp. 10 the interpolated stimulus *B* was only 20 % nearer to *C* than was the original first stimulus *A*; whereas in Exp. 9 the stimulus *B* was 34 % nearer to *C* than was the first stimulus *A*.

occurrence of the second stimulus will differ according as the second stimulus is or is not sent in at the same point as the first. If the second stimulus is sent in nearer the muscle than the first, then at the seat of the second stimulus the propagated disturbance will begin later (by the time of conduction between the two seats of stimulation) than it began at the seat of the first stimulus. Consequently the interval between the first and second stimuli will with separate points of stimulation be in effect less than when the stimuli fall on the same spot. This means that unless the time of conduction between the points of stimulation is known it is not possible by a single pair of observations to determine whether the delay of the response to the second stimulus is the same whether the stimuli fall on the same or on different parts of the nerve; for it will not be possible to ensure that the second stimuli shall in the two cases catch the first propagated disturbance in the same stage of its development. I thought the best way of getting round this difficulty would be to repeat each kind of observation with a large number of time intervals between the stimuli. If this is done it becomes possible to plot out, as was done in Figs. 6 and 10 above, the time of occurrence of the second response over a wide range of intervals between stimuli for both kinds of observation. The results of such an experiment are tabulated below (Exp. 11). Four observations were made with the second stimulus at the same point as the first, four with the second stimulus at a point on the nerve 11 mm. nearer to the muscle. The anode was in every case at the same point, namely 5 mm. further from the muscle than the seat of the first stimulus. The times of occurrence of the second response are given twice over for every observation made. In Table *A* the beginning of the second response is measured directly from the first apparent divergence of the combined from the single curve. In Table *B* the point actually measured is the time of maximum P.D. of the second response, and the time of beginning of the response is found by subtracting ·0022 sec. which is the observed interval between the beginning and culmination of a normal response.

Whether Table *A* or *B* is taken as the truer account of the times of occurrence of the second response one fact stands out clearly. When the two stimuli are sent into separate points on the nerve, as well as when they are sent in together, the delay of the second response increases in the familiar way with the earlier incidence of the second stimulus. The values obtained for the delay are not identical in the two cases, as may be seen if for example those given in Table *B* I

and II are plotted together on the same scale of time. But it must be remembered that, for reasons which have been given already, the time intervals between the stimuli for observations *E* to *H* are in effect shorter than those given in the table. If we wish to represent truly the relation of the second stimulus to the previous propagated disturbance by which delay and modification are being produced, we must make

Exp. 11. Gastrocnemius-sciatic preparation. Frog. Temp. = 17·5° C.

A. *Time of apparent commencement of second response measured directly.*

I. With second stimulus at same point as first.

Obs.	Time first stimulus to second stimulus	Time first stimulus to beginning of second response	Time second stimulus to beginning of second response
A	·0021 sec.	·0074 sec.	·0053 sec.
B	·0030	·0075	·0045
C	·0042	·0077	·0037
D	·0067	·0095	·0028

II. With second stimulus 11 mm. nearer to muscle than first.

E	·0015	·0077	·0062
F	·0029	·0072	·0043
G	·0042	·0075	·0033
H	·0064	·0089	·0025

B. *Time of commencement of second response calculated from time of its maximum* P.D.

I. With second stimulus at same point as first.

A	·0021	·0069	·0048
B	·0030	·0070	·0040
C	·0042	·0074	·0032
D	·0067	·0093	·0026

II. With second stimulus 11 mm. nearer to muscle than first.

E	·0015	·0069	·0054
F	·0029	·0067	·0040
G	·0042	·0074	·0032
H	·0064	·0084	·0020

allowance for the time of conduction of that first disturbance from its place of origin to the seat of the second stimulus. The length of nerve intervening was 11 mm. If we assume the rate of conduction in the nerve to have been 18 mm. per sec., the time occupied will have been ·0006 sec. For observations *E* to *H* we must therefore subtract this time from the tabulated interval between the stimuli. By this process the following values are obtained if we take the times given under Table *B*.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response
E	·0009 sec.	·0069 sec.
F	·0023	·0067
G	·0036	·0074
H	·0058	·0084

When this correction has been made the delay of the second response is found to agree in a remarkable way whether the second stimulus does or does not fall on the same part of the nerve as the first. This is made clear by Fig. 18 in which the two sets of observations are plotted side by side. The values are taken from Table *B*. The supposed time of conduction (·0006 sec.) has been subtracted from the time between stimuli in observations *E* to *H*. Observations made with stimuli at the same point are shown as dots enclosed in triangles; those with stimuli at different points as dots enclosed in circles. All the observations lie close to the broken curve. The same agreement may be seen if the values from Table *A*, which were obtained by an independent method of measurement, are treated in the same manner.

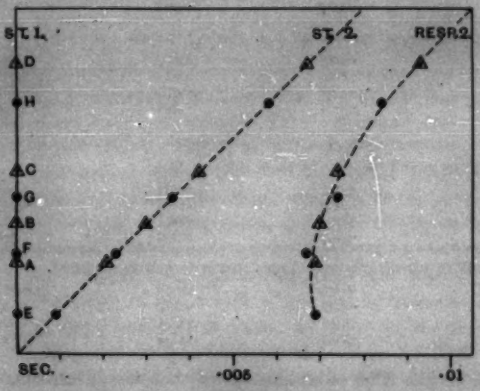

Fig. 18.

These experiments point again to the conclusion that the delay in the response to a second stimulus is due, within the limits of accuracy of the methods here used, entirely to the propagated disturbance set up by the former stimulus, and not at all to the stimulation fatigue resulting from the passage of the former exciting current.

Still one more method of experiment on this question seemed to me desirable. I have referred already to Gotch's account of experiments in which responses to an adequate stimulus showed increased delay when an inadequate stimulus had previously traversed the tissue. I

attempted to repeat this observation on muscle. A photographic record was made in the following way. A sartorius muscle was connected to the capillary electrometer in the usual manner, the exciting electrodes being on the nerve-free pelvic end, and the leading-off electrodes on the middle and tibial end. At the first exposure of the photographic plate the muscle was excited with a single maximal stimulus (*B*). At the second exposure the stimulus *B* was again sent in, preceded at an interval of ·002 sec. by a stimulus (*A*) which was just subliminal. The stimulus *A* was adjusted in strength by finding the threshold for contraction of the muscle and moving the secondary coil away 1 mm. further from the primary. In the experiments made the threshold was at about 10 cm. coil distance. From calibration of the coils used it appears that a movement of 1 mm. in this region of the inductorium means a change of strength of current by approximately 5 %. The photographic record showed that the response to stimulus *B* alone and the response to stimulus *B* preceded by *A* had traced over precisely the same course. It would have been easy in these superposed records to measure a displacement as small as 0·2 mm. between the two exposures. With the speed of plate used this would mean ·0004 sec. Four observations were made by this method at 9° C., one at 14° C., one at 16·3° C., and three at 17° C. Two muscles were used for the observations. In none of the photographic records can I trace any divergence between the response to *B* alone and the response to *B* preceded by *A*. It seems clear that in muscle the response to an adequate stimulus is not delayed to a measurable degree by the passage of a previous inadequate stimulus.

The three methods of experiment which I have used lead me to the same conclusion, that the delay in the response to a second stimulus is wholly due to the propagated disturbance set up by the first stimulus, and not at all to any localized "stimulation fatigue" which may be produced. This is so whether the muscle is excited directly or through its motor nerve.

VI. *Remarks on the application of the results obtained.*

Up to this point my experiments have shown that the occurrence of a delayed response to a second stimulus is common to many tissues, and is due, in muscle at any rate, entirely to the temporary modification of the tissue by the passage of the propagated disturbance.

The value of this result seems to me to lie in its possible contribution to two of the difficult problems with which the physiology of the excitable tissues has to deal. In attempting to investigate the nature of the nervous impulse, or of the corresponding propagated disturbance in muscle, we are always hampered by the paucity of the measurable changes which that disturbance brings in its train. Up to now the change of electric potential has been the sole recognisable accompaniment of the propagated disturbance. By the delay of the response to a second stimulus we can now measure the duration of a modified state which is left in the wake of the disturbance. Some experiments made by another investigator in this laboratory and shortly to be published have given more definite quantitative evidence than existed previously to show that the refractory period of muscle and nerve is also a consequence of the propagated disturbance alone. We begin therefore to bring the propagated disturbance more closely within the grasp of quantitative experiment. The next step must obviously lie in the direction of determining more precisely the relation between those several phenomena which go hand in hand with the propagated disturbance.

The results described in this paper must also be taken into account in any consideration of the way in which the effects of successive stimuli sent into a tissue may interfere one with another. It is to the interference of successive stimuli that we must look, according to the recent hypothesis of Fröhlich, for an explanation of the phenomena of inhibition[1]. It will be necessary therefore to enquire whether that hypothesis accords completely with the facts which have here been described. If I have understood the hypothesis rightly it supposes inhibition to result from the setting up of a new refractory period by a stimulus falling within the refractory period which succeeds a previous stimulus. My experiments indicate that a stimulus falling within the refractory period of a previous one does not set up at any rate that state of modification which causes the delay of a second response, a state which we have seen to be the consequence of the propagated disturbance. We have indications that the refractory period is probably an accompaniment of the same disturbance. This is enough to suggest the need of rigid experimental proof that a stimulus falling within the refractory period of a previous stimulus does in fact set up a new refractory period of the same nature as that within which it fell. Such a proof, if it exists, must form the very foundation of the hypothesis.

[1] *Ztschr. f. allg. Physiol.* IX. p. 55. 1909.

I might point to other ways in which the results described in the present paper bear on the question of interference between the effects of successive stimuli. But I prefer to leave the whole matter over until my own experiments have advanced a step further.

VII. Summary.

Photographic records of the electric response of the gastrocnemius muscle of the frog to two stimuli sent into the sciatic nerve have been made and analysed by the usual method. The curves relating time to potential difference show that the response to a second stimulus falling soon after the end of the refractory period commences after a delay which may be many times as great as the delay of the first response. Attempts have been made to correct for the many possible sources of error involved in measurements of this kind.

Similar observations have been made on the sartorius muscle of the frog excited directly. These confirm my previous observations on the same tissue in so far as they also show the second response to a stimulus falling soon after the end of the refractory period to be greatly delayed.

On the ventricular muscle of the frog observations of a like kind have been carried out by two methods. In one method the Stannius preparation was used, and the ventricle was excited by two stimuli following one another at various intervals. In the other method single stimuli were sent into a beating ventricle at various times after a natural beat. Both these methods show that the electric response to a stimulus falling soon after the end of the refractory period is subject to great delay.

In the simpler tissues used, the directly excited sartorius and the ventricular muscle, it is found that second stimuli falling within a certain range of time after the end of the refractory period provoke responses at a fixed interval after the first stimulus. This is the phenomenon of the irresponsive period which I have previously described. But there is an important modification of my original statement of that phenomenon rendered necessary by the greater range of these later experiments. I originally stated, in consequence of using too few intervals between stimuli in my earlier experiments, that second responses beginning later than the irresponsive period exhibited no abnormal delay. I now find that such later responses are more delayed than the response to a single stimulus; their increased delay disappears

only gradually as the second stimuli become later. This agrees with the recent observations of Gotch on nerve.

The phenomena of delay in a more complex case such as that of muscle excited from its motor nerve exhibit further peculiarities for which reference must be made to the more detailed part of this paper.

Experiments have been carried out by three independent methods in order to determine whether the modification of the tissue responsible for the increased delay of a second response is caused by the direct local action of the previous stimulating current, or by the passage of the previous propagated disturbance over the tissue, or by both. The methods used are:—

(1) To determine whether a stimulus falling within the refractory period of a previous one, and therefore setting up no propagated disturbance, can alter the amount of the increased delay.

(2) To send the stimuli provoking the second response into a part of the tissue not traversed by the previous stimulating current, and to determine whether the delay is the same as when the two seats of stimulation coincide.

(3) To enquire whether a preceding stimulus of just subliminal strength produces any delay in the response to a succeeding adequate stimulus.

The experiments made in these various ways agree in showing that in the tissues used the increased delay is, within the limits of measurement available, due entirely to a modification of the tissue by the previous propagated disturbance, and not at all to any direct effect which the preceding current used for stimulation may have.

A similar conclusion may be drawn from the observed fact that in cardiac muscle all the usual features of the delayed second response are reproduced if the first response is a natural one, when of course the second stimulus falls on a tissue not previously subjected to an electric current. These results are not in conformity with the conclusion drawn by Gotch for the case of nerve, that "stimulation fatigue" (the local effect of the stimulating current) is one important factor in causing the delay.

VIII. Appendix.

In these tables the time and potential difference values are given for those analysed electrometer curves which are used in the text but not shown graphically. In order to save space the data are tabulated only for those parts of the curves which are required for determining

the time of commencement or of maximum P.D. of the second response. Exps. 2, 4, and 5 are made by the method of single exposure, and require a control curve showing the response to the first stimulus alone for comparison with the combined responses which are tabulated. In Exp. 2 the control for *DEF* is *A*, which is given graphically in Fig. 3, the control for *G* and *H* is the first part of *H*. The controls for Exps. 4 and 5 are given in the tables. Exps. 3 and 11 are made by the method of superposed photography, so the analysed curves for both responses are given under every observation. The P.D. is given in arbitrary units. The number of units equal to ·01 volt is for Exp. 2, 35, Exp. 3, 27·8, Exp. 11, 22.

Exp. 2.

Time from zero	P.D. E	P.D. G	P.D. H
·0033 sec.	st 1	...	...
·0070	st 2	...	...
·0009	...	st 1	..
·0074	...	st 2	...
·0040	...	...	st 1
·0187	...	...	st 2
·0081	...	−56·6	+66·3
·0087	...	−48·8	+88·8
·0094	...	−38·2	+79·2
·0100	−32·4	−19·1	+4·9
·0106	−68·6	+3·4	−48·0
·0112	−53·8	+96·9	−59·1
·0119	−35·0	+163·5	−47·1
·0125	−12·9	+208·9	−37·5
·0131	−3·5	+193·8	−25·5
·0137	+6·4	+13·9	−16·5
·0144	+11·6	−139·6	−11·8
·0150	+1·7	−150·3	−6·0
·0162	−19·5	−94·0	−3·5
·0175	−9·1	−22·4	−1·4
·0200	−1·2	−4·7	−0·5
·0212	...	...	0
·0219	...	...	+10·8
·0225	...	...	+82·4
·0231	...	...	+115·4
·0237	...	...	+136·9
·0244	...	...	+134·2
·0250	...	...	+8·6

Exp. 3.

	P.D.					
	D		E		F	
Time from zero	1st resp.	2nd resp.	1st resp.	2nd resp.	1st resp.	2nd resp.
·0030 sec.	st 1	...	...	...	...	...
·0068	...	st 2	...	...	...	...
·0012	...	...	st 1	...	...	...
·0063	...	...	...	st 2	...	...
·0007	...	...	...	...	st 1	...
·0079	...	...	...	...	...	st 2
·0081	...	...	+69·6	...	...	...
·0087	...	...	+38·2	...	−6·6	...
·0094	+88·8	...	+4·1	+5·0	−56·4	...
·0100	+69·9	...	−53·3	−27·9	−74·6	...
·0106	+25·7	...	−73·1	−27·2	−62·9	−47·8
·0112	−16·3	−11·7	−53·2	+7·8	−32·1	+5·1
·0119	−80·7	−31·1	−35·4	+52·5	−18·9	+40·3
·0125	−74·3	−3·4	−25·0	+75·6	−16·5	+99·2
·0131	−54·9	+15·9	−15·9	+77·4	−12·0	+104·1
·0137	−33·3	+29·5	−10·0	+37·6	−9·1	+96·4
·0144	−22·2	+26·3	−9·2	+12·6	−8·5	+81·4
·0150	−14·2	+10·3	...	...	...	...

Exp. 4.

		P.D.			
Time from zero	Control single response	B	C	D	E
·0030 sec.	st 1	...	...	...	...
·0012	...	st 1	...	...	...
·0064	...	st 2	...	...	...
·0087	...	...	st 1	...	...
·0112	...	...	st 2	...	...
·0030	...	...	...	st 1	...
·0130	...	...	...	st 2	...
·0040	...	...	...	...	st 1
·0165	...	...	...	...	st 2
·0175	+15·3	−0·5	+20·1	...	...
·0187	+5·6	−11·7	+9·3	...	...
·0200	−5·2	−11·1	+0·6	...	...
·0206	−9·1	...	...	...	...
·0212	−13·7	−5·6	−7·2	...	...
·0219	−16·7	...	...	...	...
·0225	−15·5	+3·9	−12·4	...	...
·0231	...	...	...	...	...
·0237	−9·3	+14·8	−3·5	+6·3	...
·0244	...	...	...	+14·9	...
·0250	−4·4	+22·1	+8·9	+27·7	+5·2
·0256	...	...	...	+33·7	+11·2
·0262	−2·1	+18·6	+21·2	+35·0	+16·7
·0269	...	...	...	+33·9	+24·2
·0275	−1·1	+10·8	+25·6	+30·4	+31·7
·0281	...	...	...	...	+34·7
·0287	...	...	+19·3	...	+37·4
·0294	...	...	...	...	+35·3
·0300	0	+4·7	+13·3	...	+30·6

Exp. 5.

Time from zero	Control single response	P.D. A	B	C	D	E
0 sec.	...	st 1	...	...	...	...
·0018	...	st 2	...	...	...	...
·0015	...	...	st 1	...	...	...
·0045	...	...	st 2	...	...	...
·0020	...	...	...	st 1	...	...
·0059	...	...	...	st 2	...	...
·0015	...	...	...	...	st 1	...
·0061	...	...	...	...	st 2	...
·0010	...	...	...	...	...	st 1
·0083	...	...	...	...	...	st 2
·0075	+36·1	+48·1	...	+5·2	...	...
·0087	+15·4	+30·0	...	+30·4	...	...
·0100	+10·4	+16·3	...	+49·0	...	...
·0112	+0·1	+9·6	...	+26·4	...	...
·0119	...	+7·6	...	...	...	...
·0125	−0·4	+5·4	...	+11·8	...	...
·0131	...	+3·3	...	...	...	...
·0137	+0·9	+13·4	...	+6·4	+9·2	...
·0144	...	+16·9	...	+3·1	+16·8	+8·7
·0150	+2·7	+14·3	+7·9	+9·2	+27·6	+25·7
·0156	...	...	+11·5	+16·4	+30·3	+29·3
·0162	...	+10·4	+15·0	+25·3	+28·6	+34·5
·0169	...	...	+9·4	...	+19·5	+22·5
·0175	+2·9	+4·9	+7·5	+17·1	...	+13·3
·0187	...	...	...	+9·2	...	...
·0200	+3·6	+4·1	...	+4·6	...	...

Exp. 11. I.

Time from zero	P.D. A R 1	A R 2	B R 1	B R 2	C R 1	C R 2	D R 1	D R 2
·0040 sec.	st 1	...	...	...	...	...	...	...
·0061	...	st 2	...	...	...	...	...	...
·0040	...	...	st 1	...	...	...	...	...
·0070	...	...	...	st 2	...	...	...	...
·0087	...	...	...	...	st 1	...	...	...
·0079	...	...	...	...	...	st 2	...	...
·0015	...	...	...	...	...	...	st 1	...
·0082	...	...	...	...	...	...	...	st 2
·0100	+119·6	...	+109·3	...	+89·6	...	−54·8	...
·0106	+35·7	...	+29·7	...	+8·6	...	−28·2	...
·0112	−78·8	...	−3·8	...	−81·9	...	−18·1	−11·9
·0119	−86·3	−37·4	−61·3	−39·2	−76·4	−55·7	−7·5	+52·6
·0125	−60·1	+43·8	−56·4	+35·0	−30·8	+54·7	−4·3	+105·9
·0131	−25·3	+94·0	−29·7	+103·3	−17·3	+113·5	−3·0	+115·9
·0137	−15·6	+90·1	−19·6	+98·6	−10·3	+111·9	−2·6	+94·0
·0144	−6·3	+19·2	−10·0	+75·7	−7·0	+88·0	...	...

Exp. 11. II.

	P.D.							
	A		B		C		D	
Time from zero	R 1	R 2	R 1	R 2	R 1	R 2	R 1	R 2
·0011 sec.	st 1	...	...	...	...	...	...	...
·0026	...	st 2	...	...	...	...	...	...
·0040	...	...	st 1	...	...	...	...	...
·0069	...	...	...	st 2	...	...	...	...
·0046	...	...	...	...	st 1	...	...	...
·0088	...	...	...	...	...	st 2	...	...
·0028	...	...	...	...	...	...	st 1	...
·0092	...	...	...	...	...	...	...	st 2
·0081	−37·5	...	...	...	...	...	...	...
·0087	−76·7	...	...	...	...	...	...	...
·0094	−63·9	+6·9	...	...	...	...	...	...
·0100	−39·6	+80·6	...	...	...	...	−53·0	...
·0106	−11·4	+91·2	+9·7	...	...	...	−89·4	...
·0112	−6·1	+10·9	−66·9	...	+104·1	...	−78·2	...
·0119	...	...	−83·5	−3·0	−52·1	...	−33·2	−28·5
·0125	−3·7	−67·4	−31·5	+71·6	−108·0	−82·7	−14·6	+36·5
·0131	...	...	−15·2	+91·5	−63·8	+15·8	−7·4	+111·5
·0137	−3·7	−25·3	−11·3	+85·3	−24·3	+113·0	−3·3	+121·8
·0144	...	...	...	...	−12·9	+115·2	−3·1	+97·7
·0150	...	...	...	...	−6·5	+81·5	...	...

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ON GASTRIC SENSATION.

By F. R. MILLER, M.A., M.B.,
Demonstrator of Physiology, University of Toronto.

(*From the Physiological Laboratory, University of Toronto.*)

Our knowledge concerning gastric sensitivity is derived from a small number of researches, partly experimental and partly clinical.

The experiments on animals have consisted in applying some form of stimulation to the stomach and then observing the various reactions induced by this means. The action of local emetics ($CuSO_4$ and tartar emetic) was studied by Openchowski[1], who found that the afferent paths for exciting vomiting in this manner are contained in the vagi.

The sensory innervation of the peritoneal surfaces of the stomach was studied by Ducceschi[2], who tested the effect of electrical and mechanical stimulation on the respirations and general reactions of the animals. His results led him to infer that, whilst sensory stimuli are conducted from the entire outer surface of the stomach by both the vagi and the splanchnics, those passing by the vagi are alone capable of exciting vomiting.

Head[3] noted that the areas of cutaneous tenderness accompanying gastric disorders correspond with the distribution of the 7th, 8th and 9th dorsal nerves, but the work of Ducceschi has as yet alone yielded experimental evidence regarding the importance of the spinal nerves in the sensory innervation of the stomach.

Hertz, Cook, and Schlesinger[4], in a recent study in man, observed that the lower end of the œsophagus was considerably more sensitive than the mucous membrane of the stomach. In investigating the sensitivity of the gastric mucosa it is, therefore, essential to confine the action of the emetic substances strictly to this surface. Whether or not

[1] *Arch. f.* (*Anat. u.*) *Physiol.* p. 554. 1889.
[2] *Arch. di Fisiol.* p. 521. 1904—5.
[3] *Brain*, xvi. p. 66. 1893.
[4] This *Journal*, xxxvii. p. 481. 1908.

any precautions of this kind were taken by Openchowski in the research already mentioned it is impossible to state, for no actual experimental details are furnished and only an epitomised account is given of his results.

The present research was, therefore, undertaken at the suggestion of Professor Brodie with the idea of making a more extensive study of gastric sensation and especially of the paths taken by sensory impulses from the mucous surface of the stomach.

In selecting a form of stimulation for the gastric mucosa, the faradic current appeared the most desirable. I found, however, that neither the bipolar nor the unipolar method of stimulation, when applied to the mucous surface, was capable of inducing vomiting and, after testing a number of local emetics, I finally resorted to warm mustard solution as yielding the most satisfactory results.

The method of procedure in the experiments with mustard was as follows: the animal (cat) was anæsthetised with A.C.E. mixture and ether. The abdomen was opened in the middle line, and the stomach drawn out and protected by cloths wrung out of warm saline solution. The dorsal and ventral branches of the vagus were then isolated on the œsophagus just below the diaphragm and a ligature tied tightly around the œsophagus underneath the nerves. The pylorus was also firmly ligatured. An opening was made in the ventral surface of the fundus and a glass tube tied in. The other end of the tube was connected by a piece of rubber tubing with a small funnel. The stomach was emptied and thoroughly washed out. The mustard solution was then introduced. Almost immediately the respirations were increased in rate and force and tongue and swallowing movements, accompanied by salivation, were induced, whilst after an interval of 1—3 minutes, typical vomiting movements ensued. This result may be obtained several times in succession, the stomach being washed out after each experiment.

It will be seen that under these experimental conditions no actual discharge of the stomach contents is possible, and the occurrence of the vomiting reflex is indicated merely by the characteristic contractions of the diaphragm and abdominal muscles. The term vomiting will, accordingly, be employed in this special sense in the remainder of this paper.

In order to determine the afferent paths involved in this vomiting reflex the experiment was carried out as already detailed but, in addition, the splanchnics were isolated on both sides. Vomiting was

then excited by mustard. The splanchnics were next divided and it was found that vomiting could still be induced. The vagi were then divided on the œsophagus and it was observed that reflex vomiting was now no longer possible. This has been confirmed in several experiments.

In other similar experiments it was found impossible to induce vomiting after division of the vagi, the splanchnics alone remaining intact. We may, therefore, conclude that the vagi alone conduct from the gastric mucosa the impulses which are capable of exciting vomiting.

For the purpose of securing tracings of the vomiting movements, the operation on the stomach was performed on a cat anæsthetised with urethane. The respirations were recorded by causing the animal to breathe into a large bottle connected with a tambour. In Fig. 1 is shown the result of introducing a 5 p.c. solution of mustard into the stomach.

Fig. 1. Record of respirations in cat. Vomiting movements begin at *V*. Signal shows introduction and removal of mustard. Time in seconds.

The respirations are seen to become considerably quicker and deeper and vomiting occurs in 1 min. 54 secs. The vomiting movements take place with greater frequency than the preceding respirations, but become considerably slower after the removal of the mustard from the stomach. Following the vomiting there is a brief interval of rest, after which the breathing is gradually resumed.

It is known that stimulation of the trunk of the vagus may cause vomiting, but the effect of stimulating the branches to the stomach does not appear to have been investigated. On trial it was found that excitation of both the dorsal and ventral branch was effective.

In Fig. 2 is shown the result of exciting the central end of the dorsal vagus with the faradic current. The respirations, which were recorded in the usual manner, become deeper during the stimulation, and some imperfect attempts at vomiting occur, but complete vomiting does not take place until after the excitation. Powerful vomiting sometimes, however, appears during the actual course of the stimulation. The respiratory excursions are considerably more extensive after, than before, the period of vomiting.

Fig. 2. Effect of stimulating dorsal vagus. Vomiting occurs at *V*. Distance of secondary 10 cm. Time in seconds.

Frequently the nerves must be subjected to several excitations before vomiting is elicited, and in these cases a deepening of the respirations is often the only visible evidence of the stimulation. This is seen in Fig. 3, from a later part of the same experiment shown in Fig. 2, after the irritability of the nerves has become somewhat reduced. In other cases a few vomiting movements take place as a result of the first stimulation, whilst a succeeding brief excitation suffices to provoke powerful vomiting.

It was next necessary to ascertain by which vagus trunk in the neck the impulses travel respectively from the dorsal and ventral vagal branches at the lower end of the œsophagus.

In order to determine this point each branch of the vagus was stimulated, whilst the vagi in the neck were successively cooled. The effect of these procedures on the vomiting reflex was meantime noted. The results indicate that, whilst in some cases the excitation passes from the dorsal branch exclusively up the right vagus, and from the ventral branch up the left vagus, in many instances impulses from each stomach nerve are transmitted by both vagi.

Fig. 8. Effect of stimulating dorsal vagus. Vomiting occurs at *V*. Distance of secondary 7 cm. Time in seconds.

The final point to be determined in tracing the further course of the impulses up the vagi was which of the rootlets belonging to the 9th, 10th and 11th cranial nerves convey the excitation to the medulla. The lower rootlets were first exposed and vomiting was induced by stimulating the central ends of the vagal branches to the stomach. The ascending rootlet of the 11th nerve was then divided on each side. Stimulation of the vagus nerves again excited vomiting. The impulses from the stomach causing vomiting are, therefore, not conveyed to the medulla by these rootlets.

As the exposure of the upper rootlets involved the removal of a portion of the cerebellum considerable shock invariably resulted, and hence it was found impossible to excite vomiting by the action of

mustard. Excitation of the gastric nerves was, however, not infrequently effective. After section of the left vagus in the neck stimulation of the ventral branch of the vagus caused weak vomiting, whilst stimulation of the dorsal vagus caused more powerful vomiting. The upper rootlets on the right side were next divided and it was found that under these circumstances vomiting could no longer be excited by stimulating either gastric nerve. Although, in the experiments so far performed, the upper rootlets have been divided only on the right side, it is reasonable to suppose that the α group of rootlets transmits, on both sides, the afferent impulses which excite vomiting.

It was stated earlier that considerable salivation is invariably associated with the vomiting produced by the action of mustard in the stomach. As previous observers[1] have shown that stimulation of the vagus in the neck causes reflex salivary secretion, I excited the vagal branches to the stomach, and obtained a very definite flow of saliva in a cannula inserted in the submaxillary duct of an animal anæsthetised with chloroform and urethane.

The results so far described show the significance of the vagi as afferent gastric nerves, and I now undertook a number of experiments with the object of demonstrating, if possible, whether any similar sensory function might be attributed to the splanchnics.

With this object in view the œsophagus was divided just below the diaphragm in an animal (cat) anæsthetised with urethane and the pylorus was ligatured. A tube was tied into the opening in the cardia of the stomach and the respirations were recorded in the manner already described. Under these conditions all possible branches of the vagi to the stomach are divided, whilst most, if not all, of the sympathetic connections are retained. It was found, however, that neither the introduction of strong mustard solution nor of bromine water into the stomach caused any alteration in the respiratory movements.

In other similar experiments a cannula was tied in the submaxillary duct, but mustard solution and bromine water failed to excite any reflex flow of saliva.

I have thus been unable to prove that sensory impulses of any kind are conveyed by the splanchnics from the mucous surface of the stomach, and cannot, therefore, add any experimental confirmation to the conclusions of Head.

[1] Cp. Langley, *Schäfer's Text-Book of Physiology*, vol. i. p. 482. 1898.

SUMMARY.

1. Vomiting induced by the action of a local emetic like mustard is prevented by division of the vagi.

2. Stimulation of the central ends of the vagal branches to the stomach causes reflex flow of saliva and vomiting.

3. Experiments so far performed indicate that the excitation causing vomiting is conveyed to the medulla by the a group of rootlets.

4. I have been unable to demonstrate that the splanchnics transmit sensory impulses of any kind from the gastric mucosa.

It gives me pleasure to thank Professor Brodie for his very kind supervision of this work.

THE ACTION OF ASPHYXIA UPON THE SPINAL ANIMAL.

By G. C. MATHISON, *Beit Memorial Research Fellow.*

(*From the Institute of Physiology, University College, London.*)

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Introduction. Kaya and Starling[1] have established the fact that the main effects of asphyxia on the spinal animal are due to lack of oxygen. It has been abundantly shown that one effect of lack of oxygen upon the metabolic processes is the production of lactic and similar acids, and it is to the effect of these acids, either circulating in the blood or accumulating in the nerve centres, that the exaggerated respiratory movements seen during oxygen lack are attributed by many physiologists.

The main object of the present investigation was to observe and compare the effects of oxygen lack, the injection of weak organic acids, such as lactic acid, and the inhalation of carbon dioxide, another weak acid, upon some of the spinal nervous mechanisms.

The effects of asphyxia, produced by cessation of artificial respiration, upon the spinal cord have been investigated by numerous observers.

Kowalewsky and Adamuk[2] showed that the rise of pressure in asphyxia in curarised cats and dogs still occurred when the cord was cut across, and to some extent even when the splanchnic nerves also

were cut. They attributed the rise of pressure to contraction of the vessels mainly from stimulation of the spinal centres, but concluded also that peripheral excitation of the wall of the vessels by asphyxial blood played a part.

Schlesinger[20] showed that in the rabbit with cord cut across, asphyxia excited a rise of blood-pressure. Luchsinger[8] directed his attention to skeletal muscle as well as to circulatory phenomena. He showed that convulsions similar to those which had been described in 1857 by Kussmaul and Tenner in the intact animal, occurred in the spinal animal during asphyxia produced by compression of the aorta, and he concluded that these convulsions were due to a general stimulation of the spinal cord by the products of metabolism of asphyxia. He also noted various visceral phenomena such as evacuation of the bladder and increased intestinal movements. He attributed the rise of blood-pressure to stimulation of the spinal vaso-motor centres, though he thought that after very prolonged compression of the aorta—fifteen minutes and upwards—a contraction of the vessels occurred due to the action of asphyxial blood on the vessel wall.

Konow and Stenbeck[4] working on curarised rabbits with spinal cords transected, obtained a rise of arterial pressure after stopping artificial respiration. They compared the rise in intact animals and spinal animals, and came to the conclusion that the longer latent period in the spinal animal was due to the fact that the accumulated products of asphyxia had to reach a considerable concentration before they affected the less excitable spinal centres.

A more complete investigation of the question was carried out by Landergren[9].

From a comparison of results in intact animals and in animals with spinal cord transected he came to the conclusion that the spinal vaso-motor centres were excited during the middle of his third stage of asphyxia, while the "Krampfcentrum," which he apparently located in the spinal cord, is excited at the end of his second stage. The stimulation of spinal centres began during the stage of expiratory dyspnœa and reached its maximum during the preterminal pause. In a few of his experiments he administered hydrogen to produce asphyxia, but in others pure carbon dioxide, so that there was no attempt in his experiments to dissociate the parts played in asphyxia by oxygen lack and excess of carbon dioxide.

Asher and his co-workers[5,7], employing anæmia by obstruction of the aorta and ligation of the subclavian arteries as a method of asphyxiating

the cord, showed that the rise of blood-pressure during asphyxia in the spinal animal was abolished if the obstruction had been continued so long that faradism of the cut end of the cord was ineffective. Even if they increased the tone of the blood vessels and raised the arterial pressure by injection of saline, no rise of pressure took place during asphyxia.

The spinal cat, decapitated according to Sherrington's method[8], affords an experimental preparation much superior to any employed by previous observers. On this preparation he observed that the rise of blood-pressure in asphyxia is a practically constant phenomenon whether curari is given or no, that a certain degree of asphyxia favours the elicitation of the scratch reflex and that in this condition the scratching movement sometimes breaks out spontaneously.

Kaya and Starling[9] were the first observers who attempted to dissociate the parts played by lack of oxygen and excess of carbon dioxide in asphyxia of the spinal animal. They established the fact that the main effects of asphyxia upon the spinal cord were due to lack of oxygen, and that moderate percentages of carbon dioxide in the presence of a sufficiency of oxygen were without influence on the spinal cord. Concerning the relative parts played in asphyxia by oxygen lack and excess of carbon dioxide respectively they write as follows: "An investigation of this question shows conclusively that there is a marked difference between the behaviour of the spinal cord and the spinal cord plus medulla to these two gases." "Working with a spinal animal the administration of carbon dioxide in the presence of adequate amounts of oxygen produces no appreciable result either on the skeletal muscles or the general blood-pressure."

General methods. The experiments were carried out in spinal cats prepared, except for a few minor details, as described by Sherrington[8]. Young cats were found to yield the most satisfactory results. Their lungs are healthier and less subject to the acute œdema that often follows asphyxia, decapitation is more easily performed, with a minimum of hæmorrhage, and they require a smaller amount of anæsthetic agent. Before the decapitation was performed, the vagi and depressors and the cervical sympathetics were cut in the neck, and for convenience the carotid arteries and external jugular veins were secured. After decapitation about one centimetre of the top of the spinal cord was cut off with sharp scissors, so as to obviate the possibility of any traces of the medulla remaining. The moment decapitation was completed anæsthetics were discontinued. The more rapidly the preparation is

carried out the more active are the spinal animals, largely owing to the shorter period of the administration of the anæsthetic. The spinal animal is readily rendered acapnic, so that a nice adjustment of artificial respiration is advisable; the best criterion of this is a steady arterial pressure. In all experiments the temperature of the animals was maintained at about 36°.

For some experiments it was necessary to use curarised animals. Since the solution of curare used, a solution containing glycerine and acetic acid, was found to deleteriously affect the heart when injected into a vein, in later experiments it was injected into the cut central end of one of the carotid arteries. Quantities varying from ·5 c.c. to 1 c.c. of the 2 p.c. solution were employed. "Asphyxia" in these experiments may be taken to mean asphyxia by cessation of artificial respiration. For the production of pure oxygen lack, nitrogen, containing usually less than 1 p.c. oxygen, was administered by means of the respiration pump devised by Hans Meyer. Carbon dioxide along with excess of oxygen was administered by the same means. In view of Bohr's[26] demonstration, confirmed by Barcroft and Camis[27], of the fact that the presence of carbon dioxide influences the dissociation curve of oxyhæmoglobin for various oxygen pressures, it was thought necessary to have the curve he gives extended so as to embrace larger percentages of carbon dioxide. Mr Barcroft very kindly did this for me, and the percentages of carbon dioxide and oxygen which I have employed in these experiments have always been such that there was no interference with the efficient oxygenation of the blood and the tissues.

The acids of which the action was investigated were injected into the blood stream. It was realised that this was in all probability but a poor substitute for the production of acids within the cells of the tissues (especially the intraneuronic production) which probably occurs during oxygen lack, and that the difference might be fundamentally important, but in view of Ryffel's demonstration that lactic acid is present in considerable amounts in the blood stream after a period of violent exercise or of oxygen lack[28], and of the action of acids injected into the blood stream on the respiratory centre which Lehmann[29], following up Geppert and Zuntz' work on muscular hyperpnœa, showed, it was considered worthy of trial.

In some of the experiments the acids were injected into one of the external jugular veins, in later experiments, in order to avoid the depressant action on the heart, the injection was made into the central end of the left carotid artery. The arterial pressure in such cases was

measured in the right carotid or one of the femoral arteries. The injections were always a degree or two cooler than the body temperature of the animal.

The effects of acids were controlled in all cases by injecting an equal amount of "normal" saline solution or of solutions containing an equivalent amount of the sodium salt of the acid employed. In view of the fact that solutions whose osmotic pressure differs greatly from that of the body fluids act upon nervous centres, the solutions used were kept as far as possible isotonic with the blood. When salts containing, for example, sodium ions were injected, an attempt was made to use solutions containing the same concentration of sodium ion as the blood. For example, when NaOH was injected, $\frac{M}{6}$ NaOH or $\frac{M}{10}$ NaOH in ·4 p.c. NaCl solution was employed.

In some cases observations were made on animals with the spinal cord destroyed by passing a sound down the vertebral canal.

The circulation.

Effect of lack of oxygen.

The essential similarity of asphyxia and oxygen lack in the spinal animal was established by Kaya and Starling. In the present investigation the circulatory phenomena have been examined in greater detail by taking records of arterial pressure, heart volume and intestinal volume.

The curarised spinal cat. The changes in the blood-pressure curve have been described by Kaya and Starling. Fig. 1 is a good example of the blood-pressure change produced by administration of nitrogen to a preparation in good condition. The heart responded well to the vaso-constriction so that there was a large primary rise of arterial pressure.

A practically identical curve was yielded under the action of asphyxia on the same cat; the initial fall of pressure from the weakened action of the heart was not so marked and the rise of pressure did not come on quite so early, as the animal still had a store of oxygen in the lungs to utilise.

In less active preparations the rise of blood-pressure may be much smaller; in the average curarised animal it does not rise above 100 mm. Apart from individual variations in the animals employed,

the experimental conditions are not always equally favourable. For example, as a result of employing too much curare or chloroform, the heart is frequently unfavourably affected. In such cases the blood-pressure falls steadily until artificial respiration is resumed; then it suddenly rises to a considerable height, often to more than 150 mm. A plethysmograph record shows that a strong constriction of the intestinal vessels is present at a certain stage of the asphyxia, but the enfeebled heart fails to respond. Whenever asphyxia produces little or no effect, the same holds true for oxygen lack; the general features of the curves in the two conditions are essentially similar.

Fig. 1. *Cat.* Decapitated. The tracings of Figs. 2—14 were obtained from similar preparations. Curarised. Between the arrows nitrogen containing 1 p.c. oxygen administered. Time marker = 10 secs. in all figures except Fig. 7.

The vaso-motor changes were investigated by means of the intestinal plethysmograph, a loop about a foot long of the lower end of the small intestine being used. During asphyxia and nitrogen administration there is marked vaso-constriction at the moment when the primary rise of arterial pressure takes place.

In the experiments illustrated by Figs. 2 and 3 the primary rise of arterial pressure was small, owing probably to the condition of the heart, but the amount of vaso-constriction was very considerable. The heart rapidly began to fail and heart block came on, indicated by the increased

amplitude of the heart beat waves on the tracing[1]. Shortly after normal respiration was resumed the block disappeared, the blood-pressure rapidly rose in both cases to about 160 mm. In front of this rise of pressure the blood vessels in the intestinal loop gave way and their volume increased up to and beyond their original volume.

Fig. 2. Fig. 3.

Fig. 2. Curarised. Artificial respiration discontinued between the arrows. Upper curve = intestinal volume, lower curve = blood-pressure. Synchronous points indicated by the crosses.

Fig. 3. Same preparation as in Fig. 2. Nitrogen administered between the arrows. Intestinal volume and blood-pressure curves. The curves are correctly synchronised. Plethysmograph lever was moved down as indicated by the arrow. This figure is to be compared with Fig. 2.

Thus during both asphyxia and pure oxygen lack there is vaso-constriction. That this vaso-constriction is central in origin can readily be shown by cutting the splanchnics or by pithing the spinal cord. In this case although the tone of the vessels be raised by injecting normal

[1] This heart block has been fully described and discussed by the author in former papers. (Mathison [11]; Lewis and Mathison [12].)

saline, asphyxia or pure oxygen lack produces no rise of pressure. That the peripheral vessel wall is capable of active response can be shown by injecting 5 c.c. of $\frac{M}{10}$ $BaCl_2$ into the carotid, so as to avoid any effect on the heart; the blood-pressure at once rises, often to the neighbourhood of 200 mm.

Fig. 4. Curarised. B.P. = carotid blood-pressure. H.V. = volume of ventricles, the upstroke representing systole. Artificial respiration stopped between the arrows. Dilatation of ventricle and heart block, followed by recovery. In volume curve 5 mm. = 1 c.c. vol.

There are minor differences between the plethysmograph curves in asphyxia and oxygen lack. When artificial respiration is stopped the arterial pressure rises a little owing to an improvement in the mechanical conditions within the thorax, and as a result of this the intestinal volume increases slightly, but when the arterial pressure falls the intestinal volume continues to rise, and for a period of 30 seconds or more there is an increasing dilatation of the intestinal vessels concomitant with a falling blood-pressure. This indicates an active vaso-dilatation[1]. The blood-pressure then gradually rises and the intestinal volume decreases. When the abrupt primary rise of

[1] This early vaso-dilatation has been present in five out of six experiments in which plethysmograph curves were taken.

pressure comes on, a rapid decrease in the intestinal volume is seen; the constriction of the vessels is so great that the individual heart beats are no longer visible on the intestinal volume curve. The last part of the decrease in volume of the intestines is not entirely due to vaso-constriction, but partly to the heart failure which comes on at this point. When nitrogen is administered the initial dilatation of the vessel wall is not seen, the heart beats weaken rather rapidly and the volume curve passively follows the blood-pressure. The dilatation during asphyxia is thus apparently due to the increased amount of carbon dioxide present in the blood during asphyxia, but not present when nitrogen is administered.

Fig. 5. Curarised. Ventricular volume and blood-pressure curves. Nitrogen administered between the arrows. Dilatation of ventricle and heart block, followed by recovery.

Both with asphyxia and pure oxygen lack the dilatation of the vessels which comes on with increased pressure usually continues for some time after the pressure has fallen from its maximum; the vessels do not regain their normal tone till a few minutes later.

The changes in heart volume. These have been more fully discussed in a former paper in connection with heart block. The changes in the ventricular volume were recorded by means of a cardiometer and a volume recorder similar to those described by Jerusalem and Starling[18]. The general resemblance between the curves in asphyxia and pure oxygen lack is shown in Figs. 4 and 5.

In both conditions the heart undergoes considerable dilatation, and recovers only when respiration is resumed. In the earlier stage of asphyxia the systolic output of the heart per beat gradually increases, reaching a maximum about 30 secs. after the cessation of respiration; from that point it decreases till the onset of heart block, when with the slower rate it shows an increase. On resumption of artificial respiration the heart rapidly regains its tone and its diastolic volume decreases to less than the original, returning to the normal a minute or so later. The heart rate is greatly accelerated during the recovery of tone; it is difficult to count the rate in ordinary curves but the electro-cardiogram shows this increase in rate very well. The actual measurements of the volume changes shown in Fig. 4 are given in the following table.

During nitrogen administration no increased output is seen in the early stages; in many cases the output very rapidly diminishes, especially when the heart is enfeebled, so that in about 30 seconds it is

TABLE I. *Analysis of curve shown in Fig.* 4.

Cat. Spinal. Curarised.

Time in mins.	secs.	Carotid B.P. mm. Hg.	Ventricular rate per minute	Increase in diastolic volume in c.c.	Output of heart per beat in c.c.	Output of heart per minute in c.c.	Remarks
0	0	40	180	0	2·7	486	Artificial respiration stopped.
0	25	43	180	1·6	3·0	540	
0	45	48	192	2·8	3·2	614	Increased output.
1	0	60	216	5·4	2·7	544	
1	10	80	216	6·2	2·4	518	
1	20	80	192	7·5	7·6	306	
1	28	74	96	8·5	3·0	288	Heart block starts.
1	46	—	—	—	—	—	Respiration resumed.
1	52	66	72	10·0	2·9	209	Heart block ends.
1	59	124	204	3·5	3·9	795	
2	30	80	216	1·1	2·5	540	

less than half the original output. The increase in output during asphyxia is doubtless due to the increased tension of carbon dioxide in the blood which, as Jerusalem and Starling have shown, increases the systolic output of the heart[18].

The acceleration of the heart beat occurring about the time of the primary blood-pressure rise in asphyxia is more easily counted in electro-cardiograms. This acceleration has been referred by Luchsinger[9] and others to stimulation of the accelerator centres in the cord. Lehndorff[04], working under Prof. Starling, has shown that the heart completely isolated from the nervous system accelerates in response to a rise of blood-pressure from increased peripheral resistance. I have

found on examining a number of my curves that there was less acceleration during asphyxia where there was little or no rise of arterial pressure, whereas it was more marked when there was a large rise of pressure. A few experiments have been performed in which the upper part of the spinal cord, from which the cardiac accelerators arise, was removed. The cat was decapitated in the ordinary way and the rise of blood-pressure and change in the pulse rate produced by asphyxia were observed. Then the spinal cord above the fifth dorsal vertebra was excised. This operation brought about no appreciable decrease in pulse rate; in successful experiments asphyxia still produced a rise of blood-pressure and an acceleration of the pulse to the same extent as before the excision. Thus it appears that the spinal cord in normal conditions gives rise to no accelerator impulses, and that the acceleration of pulse rate during asphyxia is not due to stimulation of accelerator centres in the cord.

The changes in blood-pressure in the non-curarised cat are similar to those in the curarised; the primary rise of blood-pressure is sometimes extremely abrupt largely owing to the spasm of the skeletal muscles, especially those of the abdomen. The close resemblance between the effects of asphyxia and of oxygen lack is shown in the following table.

TABLE II. *Asphyxia and want of oxygen in the spinal animal.*

Cat. Spinal. Non-curarised. Heart in good condition.

	Time in mins. secs.	Arterial pressure in mm. Hg.	Rate per min.	Remarks
Asphyxia	0 0	65	138	Respiration off.
	1 10	46	—	
	1 45	50	—	Movements and spasm.
	2 10	80	156	Convulsions.
	3 20	42	72	Heart block starts.
	3 40	—	—	Respiration on.
	3 57	42	72	Heart block ends.
	4 5	135	—	
	7 5	65	144	
Nitrogen	0 0	58	144	Nitrogen on.
+2 p.c. oxygen	1 20	46	—	
4 mins. later	2 0	52	—	Movements and spasm.
	2 15	74	162	Convulsions.
	2 40	50	78	Heart block starts.
	3 5	—	—	Nitrogen off.
	3 40	51	78	Heart block ends.
	3 55	130	—	
	8 0	60	144	

Effect of injection of organic acids and of alkalies.

From the researches of Araki[95,96] and others and the recent work of Ryffel[97] it is known that an increased amount of lactic acid is present in the blood stream under certain conditions, of which oxygen lack is one of the most important. It is possible that other organic acids are produced in these conditions. As a type of such organic acids lactic acid was employed. The ordinary racemic lactic acid was used, not the dextro-compound that occurs in the body. As the acid was employed merely on account of its acidic properties, and since preliminary experiments showed that sodium lactate containing the same amount of the acid radicle produced no effect—the action of lactic acid being thus in no way specific—it was a matter of indifference whether the natural or the racemic acid was used. Occasionally the effect of other acids, such as glycollic and acetic, was tried.

It has been objected that the injection of acids into the circulation produces gross changes in the blood such as agglutination of corpuscles and conversion of a large amount of the hæmoglobin into acid hæmatin. Experiments *in vitro* showed that the proportion of acid present in the blood stream when $\frac{N}{6}$ lactic acid was injected, was not likely to produce appreciable changes of this nature. The circulatory changes produced by the injection of acids were studied in curarised and in non-curarised cats.

The curarised spinal cat. When about 5 c.c. of $\frac{N}{6}$ lactic acid are injected into the jugular vein over a period of about 15 seconds[1], a fall of pressure occurs, due largely, as cardiometer records show, to a rapid dilatation of the heart. This is succeeded in most cases by a rise of pressure, though in very many cases the rise is absent or of inconsiderable magnitude. The fact that the rise is in many cases absent, owing to the depression of the heart, probably accounts for the results of Osborne and Vincent[97], who obtained no rise of pressure on injecting 1 p.c. solutions of sarcolactic or of fermentation lactic acid. The positive result is illustrated in Fig. 6, which also shows the intestinal volume changes.

[1] From cardiometer observations on the output of the heart it is calculated that this represents less than ·2 p.c. lactic acid in the blood flowing from the heart and less than ·05 p.c. in the total blood; these percentages are much of the same order of magnitude as those found by Ryffel in the blood stream after violent exercise[97].

The vaso-motor changes. The peculiar plethysmographic curve shown in Fig. 6 has been seen in a number of experiments, and seems to depict the most characteristic result. First the intestinal volume decreases, following passively as the blood-pressure falls from the weakening of the heart; then an increase in volume takes place, followed soon by a decrease as the blood-pressure rises. After this there is a gradual and prolonged increase as the blood-pressure falls.

Fig. 6. Curarised. Upper curve = intestinal volume; lower curve = blood-pressure. Between the two large arrows 6 c.c. $\frac{N}{6}$ lactic acid were injected into the jugular vein. Fall of pressure from action on the heart, subsequent rise from vaso-constriction. The smaller arrows indicate synchronous points.

The initial decrease of volume is merely passive; the following increase is in all probability due to the direct effect of lactic acid on the vessels. Gaskell[68] showed that as little as 1 : 13,000 lactic acid produced dilatation of the frog's blood vessels through which it was perfused. According to Dixon[69], 1:10,000 lactic acid when applied to the musculature of the frog's stomach caused considerable relaxation, and stronger solutions produced the effect more rapidly; still stronger solutions, 1 : 100 lactic acid, produced on the other hand tonic contraction. The constriction of the vessels with the rise of arterial pressure is due to stimulation of the spinal vaso-motor centres. The subsequent dilatation of the vessels is probably due to the direct action of the acid on the vessel wall. A second injection of lactic acid again produces vaso-constriction, so that the dilatation cannot be the result of paralysis of the vaso-motor centres.

When the acids are injected into the arterial side their depressant action on the heart is avoided, since before they reach the heart they have to pass through the general circulation. The effect produced is shown in Fig. 7. Glycollic acid was employed instead of lactic in this particular case and the animal was not curarised, so that the connections of the arterial cannula became kinked by the animal's struggles and the heart beats are absent from part of the tracing. The effects of lactic acid on the blood-pressure when injected into the venous and into the arterial side are also illustrated in Figs. 12 and 13.

Fig. 7. Non-curarised. Blood-pressure in right carotid. 8 c.c. $\frac{N}{5}$ glycollic acid injected into left carotid. Kinking of artery due to spasm of skeletal muscles. Note absence of depressant action on heart. Time = seconds.

Alkalies. The action of alkalies is somewhat interesting. Gaskell showed that perfusion with NaOH in 1 : 10,000 solution caused constriction of the frog's blood vessels, the action being antagonistic to that of acid. If 5 c.c. of $\frac{N}{10}$ NaOH, made up with about ·5 p.c. NaCl so that the same amount of sodium ion is present as in the body fluids, are injected into the arterial stream, a considerable rise of pressure takes place, for example from 50 mm. to 110 mm. Hg. If, when this has passed off, the spinal cord is pithed and the injection repeated, much the same increase of pressure is seen, and, since the sodium ion is in normal concentration, this direct effect on the wall of the vessel must be due to the hydroxyl ion. The injections of lactic acid were controlled by the injection of equivalent amounts of sodium lactate solution, but these were found to produce no effect other than a rise of 1 to 2 mm. due to the volume of fluid injected.

Effect on the heart volume. The changes in heart volume have been observed in a number of experiments. When small doses such as 5 c.c. $\frac{N}{10}$ lactic acid are injected over a period of about 20 secs. into a jugular vein a little dilatation of the heart occurs without any great change in

the systolic output; sometimes a slightly increased systolic output is seen. The dilatation, which in a heart that is ejecting 3 c.c. of blood at each systole amounts to about 1·5 c.c., passes away in the course of a couple of minutes. When larger doses, such as 5 c.c. $\frac{N}{4}$ lactic acid, are given very great dilatation occurs, the increase in volume amounting to perhaps 8 c.c.; at the same time the systolic output is progressively lessened falling from 3 c.c. to ·5 c.c. without any period of increase; complete recovery only takes place after many minutes. If still larger doses are given the heart rapidly dilates and asystole ensues; no restorative measures are able to bring about recovery, even mechanical massage being of no avail[1].

Effect of excess of carbon dioxide.

Employing much larger percentages of carbon dioxide than those used by Kaya and Starling[10], marked changes have been seen in the circulation of the spinal cat. The circulatory changes when large percentages of carbon dioxide, mixed with an adequate supply of

Fig. 8. Curarised. Between the arrows CO_2 36 p.c. + O_2 50 p.c. administered. Rise due mainly to vaso-constriction. Time = 10 secs.

[1] The results have only been stated broadly here as the whole question of the action of oxygen lack, lactic acid and large doses of carbon dioxide on the mammalian heart, is at present under investigation.

oxygen, are administered, show considerable variation. In some cases the blood-pressure shows little change even with high percentages, 30 p.c. and upwards, of the gas, while with lower percentages there is a steady rise due to increased output of the heart. In these cases the threshold of the spinal cord for carbon dioxide is apparently very high, and it is at the same time higher than usual as regards oxygen lack and injection of acids.

The cases which give a large rise of arterial pressure will be considered first. Such rise of pressure rarely takes place with less than 25 p.c. In one case, however, a mixture containing 21 p.c. CO_2 and 70 p.c. O_2 caused the blood-pressure to fall in 50 secs. from 58 mm. to 46 mm.: three and a half minutes later the pressure had risen to 160 mm., and did not return to the normal level until over two minutes after the carbon dioxide was discontinued.

An experiment with positive results is illustrated in Fig. 8, which shows the effect of a mixture containing 36 p.c. CO_2 and over 50 p.c. O_2 on the arterial pressure. This curve illustrates the main features, an initial fall, a rise, at first gradual then more rapid, which is sustained for some time, then a further rise when respiration of ordinary air is resumed.

The analysis of this figure is as follows:

Time in mins. secs.	Carotid blood-pressure in mm. Hg.	Remarks
0 0	59	CO_2 36 p.c. + O_2 50 p.c. on.
1 0	42	
3 0	71	
4 20	189	
4 45	183	CO_2 + O_2 off.
5 0	181	
5 20	194	
5 50	209	
7 0	85	

In the same cat on another occasion 36 p.c. CO_2 produced a fall from 44 mm. to 34 mm. in one minute, in three mins. the pressure rose to 194 mm., and two mins. later, when the carbon dioxide was withdrawn, the pressure was 180 mm. In a third experiment with 46 p.c. CO_2 the pressure rose from 70 mm. to 160 mm. in 3 mins. 30 secs., and had only fallen to 156 mm. two mins. later.

The great changes in arterial pressure can only be accounted for by the presence of vaso-constriction, and since they are never seen when the cord is pithed, this constriction must be due to excitation of the spinal vaso-motor centres. The direct action of carbon dioxide on the vessel wall is, according to most observers, to cause vaso-dilatation.

Bayliss[29] in an experiment on the flow through the hind limb of a dog, where a compensator was employed to keep the head of pressure constant, found that blood saturated with carbon dioxide caused vaso-dilatation. Severini[30] also obtained dilatation with carbon dioxide. Stefani[31], perfusing through isolated organs, found that asphyxial blood had a local dilator effect on the vessel walls, and showed that the general vaso-constriction was caused through its action on nerve centres.

The behaviour of the heart during these changes of pressure will be considered later.

Fig. 9.

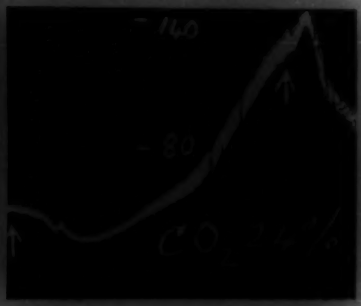

Fig. 10.

Fig. 9. Non-curarised. Between the arrows CO_2 16 p.c. + O_2 50 p.c. administered. Rise in pressure probably entirely cardiac in origin. Compare with Fig. 10.

Fig. 10. Same preparation as in Fig. 9. Between the arrows CO_2 24 p.c. + O_2 50 p.c. administered. Rise of pressure largely due to vaso-constriction; to be compared with Fig. 9.

Percentages of carbon dioxide which are below the threshold value for the spinal vaso-motor centres may nevertheless produce considerable rise in pressure. This effect is well seen with less than 20 p.c. carbon dioxide, as this often increases the output of the heart. The curves produced differ somewhat from those yielded when vaso-constriction is present, and after experience of cardiometer records one is able to judge from the curves whether the spinal vaso-motor centres have been excited or not. For example, comparison of Figs. 9 and 10 at once suggests a difference in the phenomena taking place. In Fig. 9 is shown a rise of

pressure from 60 to 90 mm., probably entirely cardiac in origin; in Fig. 10 from the same animal the rise from 42 mm. to 132 mm. is largely due to vaso-constriction. When amounts of carbon dioxide of 30 p.c. and upwards fail to excite the vaso-motor centre less rise of pressure occurs than with the lower percentages, as the heart does not beat so powerfully; frequently there is practically no increase of pressure.

The changes in heart volume. Jerusalem and Starling[28] showed that the effect of moderate percentages of carbon dioxide (2 to 8 p.c.) in the air respired was to increase the ventricular output of the mammalian heart (cat) working against a constant resistance, but that with high percentages, 12 to 20 p.c., the cardiac dilatation affects the systolic more than the diastolic volume, so that the ventricular output is diminished. They place 12 p.c. as about the outside limit at which an increase in the output per beat can be obtained. In experiments on the spinal animal with percentages between 10 and 20 p.c., some depression of the heart's activity is usually seen, but if the administration of the gas be continued the heart recovers and beats more vigorously than before, so that the blood-pressure may rise apart from any vaso-constriction.

To my surprise I found that with far larger percentages of carbon dioxide, 40 to 50 p.c., the systolic output of the heart, though at first greatly diminished, showed later a very great increase.

Apparently there is considerable difference between the action of carbon dioxide on the heart working in an isolated heart-lung circulation against a constant resistance and its action on the heart working in the whole circulation against a variable resistance. It seems probable that the explanation lies in the different changes produced in the reaction of the blood in the two conditions. In the isolated heart-lung circulation the blood does not pass through organs capable of neutralising any excess of acid which it contains; normally the lungs by turning out carbon dioxide can accomplish a certain amount in this direction, but they are unable to exercise this function when the alveoli already contain large percentages of carbon dioxide. Thus upon the blood itself falls the brunt of regulating its own reaction, and though the chemical composition of this fluid is such that it is capable of neutralising a considerable quantity of acid without greatly altering its hydrogen ion concentration, it cannot do so indefinitely (cf. Henderson[29]). With large percentages of carbon dioxide there is probably a marked increase in the hydrogen ion concentration. On the other hand, if the blood is flowing through the whole circulation changes in composition are soon set right by the action of organs such as the liver and kidneys, so that

the hydrogen ion concentration is kept fairly constant. It is only when the organs fail in their function that changes in the composition of the blood persist for any length of time.

It is evident in cardiometer tracings such as that shown in Fig. 11, that the metabolic conditions in the cardiac tissues during the administration of these large quantities of carbon dioxide must be very complicated. At first the heart is greatly weakened, then some alteration in the conditions takes place—perhaps some chemical reserve is called on—and the heart greatly improves. The cardiac output per minute in the second experiment shown in Table III increased to over three times that existing at the beginning of the experiment.

Fig. 11. Curarised. Upper curve = ventricular volume, upstroke representing systole, 5 mm. = 1 c.c. vol. Lower curve = carotid blood-pressure. Between the arrows CO_2 42 p.c. + O_2 45 p.c. administered. Dilatation and increased output of heart. Analysis in Exp. 1, Table III. Gaps in tracing = 20, 40, 60, 40 secs. respectively.

The action of 42 p.c. CO_2 is illustrated in Fig. 11; the cardiometric method employed has been described elsewhere. Dilatation of the heart commences soon after the carbon dioxide is started, the systolic output of the heart is greatly diminished and the blood-pressure sinks; then

the heart commences to improve and a rise of pressure is seen. This rise is considerably greater than can be accounted for by the increased cardiac output, showing that an increased peripheral resistance from vaso-constriction is present. The pressure continues to rise and the heart improves till its output per minute is over 30 p.c. more than the original output. At this point the carbon dioxide is withdrawn and atmospheric air substituted, and the heart rapidly regains its tone, so that the diastolic volume becomes lessened and soon reaches the normal.

TABLE III.

Effect of large percentage of carbon dioxide on blood-pressure, cardiac volume, and cardiac output.

Exp. 1 shown in Fig. 11. CO_2 43 p.c., O_2 45 p.c.

Time in mins. secs.	Arterial pressure in mm. Hg.	Increase in diastolic volume in c.c.	Output of ventricle per beat in c.c.	Rate of ventricle per minute	Output of ventricle per minute in c.c.	Remarks
0 0	58	0	2·7	192	518	CO_2 on.
0 40	45	2·4	1·6	192	307	
1 10	43	2·8	1·4	192	268	
1 50	66	2·8	2·4	180	432	
2 20	100	2·4	3·0	180	540	
3 25	135	2·8	4·0	168	672	CO_2 off, air on.
3 50	140	2·8	4·1	168	688	
4 0	135	1·6	4·0	168	672	
4 40	87	−0·6	2·8	192	537	
5 0	76	−0·8	2·6	192	499	
7 0	50	0·2	2·0	180	360	
Exp. 2.	Spinal cat, curarised.		CO_2 32 p.c., O_2 57 p.c.			
0 0	36	0	1·4	162	226	CO_2 on.
1 0	24	3·6	0·8	168	134	
2 5	30	3·8	1·0	168	168	
3 0	50	3·4	1·8	174	313	
3 15	72	2·9	2·6	174	452	
3 40	95	3·1	3·2	174	556	
4 0	110	3·4	3·6	174	626	
4 35	125	3·6	3·9	168	643	
4 55	130	3·5	4·0	168	672	
5 5	—	—	—	—	—	CO_2 off, air on.
5 10	132	3·2	3·8	180	684	
5 25	141	2·2	3·4	180	632	
5 45	117	−1·4	2·2	204	449	
6 0	96	−1·4	2·0	204	408	
7 0	68	−1·2	2·0	204	408	
8 0	50	−1·2	1·5	192	288	

In most cases the volume even decreases to less than the original, showing that the tone of the heart muscle is abnormally increased. Contemporaneous with this restoration of tone in the heart muscle, the blood-pressure curve tends sharply upwards. This rise in blood-pressure is a characteristic feature of carbon dioxide curves; there is no additional increase in the output of the heart at this moment, so that the rise must be attributed to an increase in peripheral resistance. It seems probable that it is due to an increase in tone in the vessel wall after the cessation of the relaxing action of carbon dioxide—an increase in tone similar to that seen in the heart muscle.

In the second part of Table III are given the results of an experiment in which much less vaso-constriction was present. The rise in pressure from 35 mm. to 95 mm. can be accounted for by the increased cardiac output. After this the peripheral resistance increased and accounted for part of the rise to 140 mm., though the increased cardiac output would itself have produced a rise to 110 mm. In the first part of the experiment any stimulating effect of the carbon dioxide upon the centres was balanced by its direct action on the vessel walls. If the results of such experiments be plotted with the ordinates representing arterial pressure and the abscissæ the cardiac output per minute, one can see at a glance whether much vaso-constriction has been present. If the blood-pressure is proportional to the output as in the first part of Exp. 2, the curve is a straight line; when vaso-constriction ensues the curve is deflected from the straight line, the greater the vaso-constriction, the more acute is the deflection.

Heart block. In a former paper[m] I showed that heart block was not produced by carbon dioxide in the presence of adequate amounts of oxygen, while it was invariably produced by administration of nitrogen. In a more extended series I have in two experiments observed heart block during the administration of carbon dioxide—on one occasion in a decerebrate cat where a mixture containing 38 p.c. CO_2 was given, on the other in a spinal cat during the administration of 51 p.c. CO_2. In both these cases the animals were curarised; the blood-pressure was high, but not abnormally so, and the blood showed no signs of inefficient oxygenation. Measurements of the auriculo-ventricular interval show that it is frequently lengthened under the action of carbon dioxide. Thus it appears that, though the block during asphyxia is undoubtedly due to oxygen lack, it is possible to produce a depression of the conductivity of the auriculo-ventricular bundle by means of carbon dioxide.

The skeletal muscle centres.

It was pointed out in the introduction that some of the older observers attributed the general convulsions of asphyxia to stimulation of centres in the spinal cord and Sherrington's observations on spinal cats have shown how constantly this spasm of the skeletal muscle occurs during asphyxia. Kaya and Starling[33] showed that this spasm occurred invariably when nitrogen was administered, accompanying or slightly preceding the rise of blood-pressure, and Bethe[34] noticed that the movements of the hind limb of a dog with spinal cord transected were much more excitable when the dog respired an atmosphere of hydrogen. Sherrington[35] has more recently pointed out that the presence of a certain degree of asphyxia favours the elicitation of the scratch and other spinal reflexes. In the present experiments some attention has been directed to the skeletal movements.

Lack of oxygen. In the general convulsion which occurs during asphyxia or oxygen lack the muscles of the trunk contract strongly; sometimes opisthotonos, sometimes emprosthotonos, is produced. In many experiments there is at first opisthotonos, the back being strongly arched, later emprosthotonos ensues, the abdominal muscles become rigid and the neck is raised several inches from the table on which the cat is lying. Along with the opisthotonos the hind limbs are rigidly extended: the fore limbs are held extended away from the sides but they are not usually so rigid as the hind limbs. After the general convulsion is over the preparation is usually inactive and reflexes cannot be elicited until the spinal cord has been supplied with oxygen.

In a spinal cat whose reflexes are inexcitable under ordinary conditions, after a short administration of nitrogen the reflexes are readily elicited. As the animal lies supine on the table, frequently alternate flexion and extension of the foot arise spontaneously and soon develop into a complete scratching movement. By stimulating the appropriate receptive fields other reflexes, such as the extensor thrust and the tail flexion, which were inexcitable in the normal condition, can be readily evoked. When reflexes are spontaneously active during the normal condition, oxygen lack brings about a tremendous increase of activity; after 30 seconds' administration of nitrogen the spinal animal may perform a rapid succession of scratch movements, to restrain which considerable force is necessary. As a point of interest, I may mention that even in these extremely vigorous scratch movements, I have never

seen the claws protruded. As a rule the various reflex movements cease just before or at the onset of the general convulsion, the limbs and the tail become rigidly extended; the phasic condition has been succeeded by the tonic one. Occasionally after the convulsion a few scratching movements may be seen.

It is noticeable that movements of the fore limb do not occur spontaneously during asphyxia and are with difficulty elicited; this may be due partly to the fact that a large portion of the receptive field for such movements has been removed, partly to the small spinal representation of such movements, much smaller than that for the hind limb, the fore limb movements being more under the dominance of higher nervous arcs.

In view of the fact that changes in the circulation occur about the same time as the increased reflex excitability, it was thought advisable to maintain the circulation at a steady level in several experiments where the action of oxygen lack, injection of acids, and excess of carbon dioxide was tried. This was done by means of the compensator described by Bayliss, and although the blood-pressure was maintained at a steady level, the increased reflex excitability and the general convulsion occurred as usual.

Injection of acids. The effect of acids injected into the circulation by way of the jugular or the carotid resembles that produced by oxygen lack or asphyxia. Spontaneous eruption of reflexes, especially the scratch reflex, amounting to clonic convulsions, and in larger doses tonic spasm of trunk muscles and extension of the hind limbs, appear. The dose required to produce these effects was as a rule smaller than that required to bring about a rise of blood-pressure. Where 5 c.c. of $\frac{N}{6}$ lactic acid injected over a period of 15 secs. into the carotid were necessary to produce a rise of blood-pressure, 5 c.c. of $\frac{N}{10}$ acid readily produced scratching movements of the hind limbs. This does not necessarily mean that the somatic centres are more readily excited by the chemical stimulus than the visceral centres, for it must be remembered that the vaso-motor discharge has to overcome the direct dilating effect of the acid on the vessels before a rise in pressure becomes manifest; several times evacuation of the bladder has been noticed at the same time as the skeletal muscular movements although no rise of arterial pressure was seen.

In all cases the injections were controlled by injections of normal saline or sodium lactate solution, but these produced no effect. Strongly

hypertonic solutions of NaCl and solutions of KCl produced twitchings of the muscles, but these could not be construed into movements and occurred even in curarised animals. Other acids such as glycollic and acetic were efficacious in provoking movements similar to those produced by lactic acid.

Excess of carbon dioxide. The action of carbon dioxide on the skeletal muscle movements of the spinal cat appears to differ greatly from that of oxygen lack or injection of acids. Kaya and Starling[1] remarked the absence of convulsions when carbon dioxide was administered. The action of carbon dioxide upon nervous centres was long looked on as a paralysing one; Verworn and his pupils particularly adopted this view, their results being based largely upon the effect of carbon dioxide in stopping the convulsions of frogs poisoned with extremely large doses of strychnine. Where excitatory effects were met with they were ascribed to reflex excitation from the sensory nerves of the respiratory passages. When the work of Haldane and Priestley[28] emphasised the point, long ago insisted on by Miescher[29], that the respiratory centre at any rate is exquisitely sensitive to carbon dioxide, it became obvious that the action of carbon dioxide on nerve centres was not merely a paralysing one. Winterstein[27] and others who had previously held an opposite opinion, acknowledged that the effect of carbon dioxide upon the centres was excitatory and only in large doses paralytic.

The anæsthetic action of large percentages of carbon dioxide on mammals was noted by Paul Bert[30] in 1878. There are few observations on the question in which the results are not complicated by the coexistence of oxygen lack. For example Guthrie and Ryan[31] showed that in a cat suffering from strychnine convulsions relaxation rapidly took place when the animal was placed in an atmosphere of pure carbon dioxide—here of course there was lack of oxygen at the same time as excess of carbon dioxide. Hill and Flack[32] have recently pointed out the depressant action on the respiratory centre of carbon dioxide in percentages higher than 35 p.c. Though they state that in certain experiments oxygen was present in excess, examination of the figures given shows that in one of the two experiments illustrated the mixture employed contained 34·6 p.c. CO_2 and 11·6 p.c. O_2, which certainly would not ensure adequate oxygenation. With larger percentages of carbon dioxide where a depressant effect was obtained, mixtures such as 65·28 p.c. CO_2 and 16·98 O_2 were employed; here oxygenation must have been quite inadequate. In view of the paucity of experimental proof of this paralysing action of carbon dioxide, it did not

seem satisfactory to pass over the absence of convulsions in the spinal animal without further investigation. The excitability of the scratch reflex was tested in several experiments where mixtures containing 15 p.c. to 60 p.c. carbon dioxide with percentages of oxygen ranging from 60 p.c. to 35 p.c.—it is obvious that where 50 p.c. and more carbon dioxide is given it is impossible to have an equal or a greater amount of oxygen present—were administered. Apart from these special experiments the notes of all spinal experiments in which carbon dioxide had been administered were examined as to the presence or absence of movements. The results are arbitrarily grouped according to the percentage of carbon dioxide given. As the effects of these different percentages were not compared in each and every spinal animal, it will be understood that the collective comparison of effects is not a strictly accurate one.

20 *p.c. and under.* These amounts have no appreciable influence on the skeletal movements, just as they have no influence on vaso-motor centres. Where reflexes are excitable at the commencement of administration, their excitability persists.

20 *to* 40 *p.c.* In over twenty experiments I have only twice seen a spontaneous scratch reflex arise. After about 90 secs. respiration of an atmosphere containing 25 p.c. carbon dioxide and excess of oxygen, an animal that had been lying perfectly quiet developed slight rapidly repeated alternate flexion and extension at the ankle and later at the knee, but no further advance towards a complete scratch reflex was made. In another case where 26 p.c. carbon dioxide produced a fall of arterial pressure from 70 mm. to 56 mm. at the end of 50 secs., with a subsequent rise to 112 mm. at the end of 2 mins. 20 secs., marked spontaneous scratch reflexes occurred at about the end of the second minute. In this case it is impossible to exclude the effect of circulatory changes, though judging from other experiments they seem to play no part. In the remaining spinal cats no spontaneous movements occurred even where there was great vaso-motor spasm. For example, in the experiment depicted in Fig. 15, where a rise of pressure to 165 mm. took place, no movements were seen. In some of these experiments no change in the reflexes was apparent. For example, in a preparation in which the reflex movements were not spontaneous but were very active when elicited, after five minutes' administration of a mixture of 38 p.c. CO_2 and over 50 p.c. O_2 no change in activity could be observed. In about half the experiments there was a distinct depression of excitability, amounting with the higher percentages to narcosis.

Over 40 *p.c.* The effect of these large amounts of carbon dioxide is

generally a depressant one. Experiments occurred in which the normal spinal preparation was so active that continuous scratch movements of the limbs took place, and when these were restrained there was a spread to other parts of the body, and vigorous movements of trunk and neck took place—this although there was no suspicion of deficient oxygenation of the blood. The administration of 40 to 50 p.c. carbon dioxide quietened the struggles in less than half a minute; the movements became progressively weaker and soon ceased, but reappeared a few minutes after the administration of carbon dioxide ceased. With larger percentages of carbon dioxide the effect is invariably narcotic, and the narcosis may persist for a considerable time.

That this depression of excitability by carbon dioxide is not due to a *paralysis* of the spinal nervous mechanism is shown by experiments in which nitrogen and carbon dioxide were administered together. In an experiment where the reflexes had disappeared after the administration of about 50 p.c. CO_2, an atmosphere consisting of equal parts of carbon dioxide and nitrogen was administered; the asphyxial spasms came on quite as early and apparently with as great intensity as usual, the only difference from an ordinary oxygen lack being that there was more extreme depression of the heart.

Such experiments show that it is not sufficient to designate the action of these large doses of carbon dioxide a "paralysing" one; some qualification is necessary. It is difficult to see why carbon dioxide, which excites visceral and somatic nervous centres in the medulla and visceral centres in the cord, should fail to excite the spinal somatic centres. Various explanations suggest themselves, but as no conclusion has been reached it is thought better simply to present the experimental results without comment.

Excess of oxygen. In the course of these experiments there have been many opportunities for observing the effects of administration of oxygen. In no experiment have I seen any evidence in favour of the view put forward by Bethe[31] that under normal conditions the administration of oxygen lessens the excitability of the spinal centres. After five minutes' administration of practically pure oxygen to spinal cats in good condition, the reflexes showed no diminution of activity. Of course if a certain degree of asphyxia exists, as must frequently be the case in conditions such as strychnine poisoning, which several workers have selected for observing the depressant effect of oxygen, the administration of oxygen, by bringing about the complete oxidation of the lactic acid and other products of metabolism which are exciting

the nerve centres, will lessen the excitability of the spinal cord. Once these products are completely oxidised, the presence of excess of oxygen can bring about no further change in the composition of the blood, and it is difficult to conceive how it can affect the chemical conditions in the nerve centres. Haldane and Priestley's experiments, in which they observed that in man respiration of mixtures containing great excess of oxygen had no influence on the respiratory movements, show clearly that the amount of oxygen normally supplied is quite adequate to effect the necessary oxidations in the tissues, and that an additional supply of oxygen has no effect on the chemical processes within the respiratory centre. The same doubtless holds true for other centres. They further showed that there is considerable margin as regards oxygen supply, and that the percentage in the air breathed can be reduced to about two-thirds of the ordinary amount before any oxygen lack is evident[39].

Repeated and prolonged asphyxia.

Summation. It was noted earlier in this paper in connection with the effects of acids, that when one injection of acid failed to produce an effect, a second injection a couple of minutes later might be

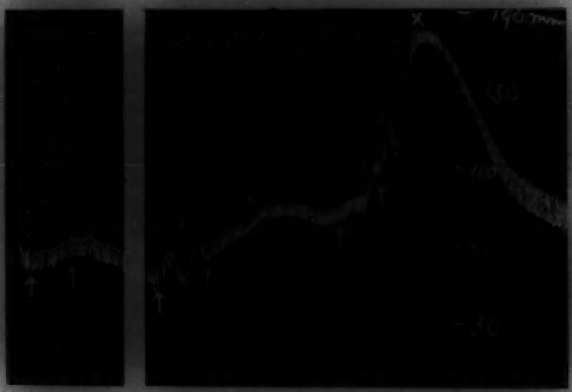

Fig. 12. Non-curarised. Blood-pressure curve from right carotid. Between the arrows during each of the periods indicated 6 c.c. $\frac{N}{7}$ lactic acid were injected into the left carotid artery. At the points marked X muscular movements or spasms occurred. Gap in tracing = 1 min. 40 secs.

effective, and a third one produce still greater result. This is true for both vaso-constrictor and skeletal muscle centres in the cord; it is an example of summation of chemical action[1].

Fig. 13. Non-curarised. Three injections of 6 c.c. $\frac{N}{6}$ lactic acid into the jugular vein during a period of 20 seconds marked by the arrows. At the conclusion of the first tracing (1) the drum was turned back, and a second injection made after 1 min. 30 secs. wait. At the conclusion of this tracing (2) after a wait of 1 min. 30 secs. a third injection was given. X indicates muscular movements. Note summation of the effect on the heart and on the blood-pressure.

In this experiment sodium hydrate and sodium carbonate had been injected sometime previously, so that there was an excess of alkali in the blood. The analysis of the curve is as follows:

Time in mins. secs.	Carotid pressure in mm. Hg.	Remarks
0 0	62	6 c.c. $\frac{N}{7}$ lactic acid into left carotid: no movements.
0 25	67	
0 45	71	
2 40	54	
2 50	55	6 c.c. $\frac{N}{7}$ lactic acid into left carotid: strong movements.
3 20	68	
4 0	88	
4 50	83	6 c.c. $\frac{N}{7}$ lactic acid. Movements and spasm.
5 20	110	
5 35	128	
5 50	176	More muscular spasms.
7 10	84	

[1] By the use of the term summation it is not intended to convey the impression that an exact parallelism exists between the condition described and the summation of nervous impulses. The acid first injected may at once be neutralised by available bases in the blood, bringing about little change in the hydrogen ion concentration of the fluids bathing the tissues, but when more acid is injected, at a certain point a sudden increase in hydrogen ion concentration takes place, and by this change in reaction of the fluids the centres are excited.

Good examples of this phenomenon are seen in Figs. 12 and 13.

In the experiment illustrated in Fig. 13, the injections were made into the venous side, so that a marked depressant effect on the heart was produced; the blood-pressure however rose higher with each succeeding injection.

It was also noticed that an injection of acid shortly after a period of asphyxia often proved effective when the same quantity before the asphyxia had produced no effect.

A similar summation of effect was seen for carbon dioxide and injection of acids; frequently a percentage of the gas which before the injection of acids was ineffective, after the injection of, for example, 20 c.c. of $\frac{N}{10}$ acid sufficiently slowly to produce no apparent effect,

Fig. 14 A. Non-curarised. First asphyxia by cessation of artificial respiration between the arrows, followed at once by oxygen 60 p.c. Gap in tracing = 1 min. 30 secs.

Fig. 14 B. 30 seconds after conclusion of A. Rise of blood-pressure due to CO_2 28 p.c. + O_2 60 p.c. shown in upper tracing. Drum turned back, and one minute later asphyxia produced by cessation of respiration between the arrows; followed at once by CO_2 28 p.c. + O_2 60 p.c. (lower tracing). The rise of pressure in the cases of oxygen after asphyxia, carbon dioxide alone, and carbon dioxide after asphyxia, to be compared.

Fig. 14 A. Asphyxia followed by oxygen. The analysis of this figure is as follows:

Time in mins. secs.	Carotid pressure in mm. Hg.	Remarks
0 0	72	Respiration stopped.
0 10	80	
0 30	86	
2 0	48	
2 50	40	
3 10	44	Convulsions and general spasms.
3 15	94	
4 0	52	
4 40	46	Respiration resumed with 60 p.c. O_2.
4 50	106	
5 10	142	
7 0	82	
7 30	70	

Fig. 14 B. Carbon dioxide, 28 p.c., with excess of oxygen. 30 secs. after experiment shown in Fig. 14 A.

Time in mins. secs.	Carotid pressure in mm. Hg.	Remarks
0 0	70	CO_2 28 p.c. + O_2 60 p.c. started.
1 1	52	
3 0	164	
3 45	162	CO_2 and O_2 off, air on.
5 0	124	
6 0	57	

One minute later. Asphyxia followed by carbon dioxide.

0 0	53	Respiration off.
1 50	30	
2 20	30	Convulsions.
2 25	72	
2 37	77	
3 40	42	CO_2 28 p.c. + O_2 60 p.c. on.
4 0	42	
4 20	80	
4 40	64	
5 20	182	
6 40	195	
9 40	150	CO_2 + O_2 off, air on.
10 10	148	
12 50	72	
15 30	50	

brought about a large rise of blood-pressure. The result shown in Fig. 14 suggests that the effects of lack of oxygen and excess of carbon dioxide may summate in a similar manner. In this experiment a spinal cat in good condition was employed. The administration of 13 p.c. CO_2 produced little change in the blood-pressure. The cat was

then asphyxiated by cessation of artificial respiration; when respiration was resumed an atmosphere containing 60 p.c. oxygen was given and the blood-pressure rose to about 145 mm. After a few minutes' wait a mixture of 31 p.c. CO_2 and 60 p.c. O_2 was administered. The blood-pressure rose in a little over three minutes to 164 mm. Five minutes after the carbon dioxide had been discontinued the blood-pressure had fallen to 53 mm. Asphyxia was then instituted and produced a change of arterial pressure very similar to that seen on the first occasion. Instead of resuming respiration with atmospheric air or oxygen, the mixture of 31 p.c. CO_2 and 60 p.c. O_2 was used; three mins. later the arterial pressure had risen from 42 mm. to 195 mm. Thus the effect of carbon dioxide following asphyxia was greater than the effect of the carbon dioxide alone, which in turn was greater than the effect of oxygen following asphyxia.

Narcosis. As a result of periods of asphyxia or nitrogen administration repeated with only short intervening intervals, of repeated injection of acids, or of prolonged action of very high percentages of carbon dioxide, the spinal animal is brought into a condition that may be termed narcosis, in which neither rise of blood-pressure nor reflex skeletal muscle movements can be elicited. Frequently when several successive injections of acids have produced increasing effects, a further injection produces much less effect and sooner or later a point is arrived at where no response is present. Asphyxia now causes neither rise of blood-pressure nor muscular movement. That the fault is with the spinal mechanisms can be shown in the case of the skeletal muscles by stimulation of a motor nerve, which produces muscular contraction: in the case of the circulation, by the injection into the arterial side of a little barium chloride solution, which at once produces a huge rise of blood-pressure. Thus the motor nerve endings and the vessel wall and heart are in sufficiently good condition; the fault lies with the centres. This spinal narcosis or inhibition may last for some time, but as a rule in about a quarter of an hour—the time varies according to the depth of the narcosis—the spinal cord is again responsive. In the case of higher centres, *e.g.* those of the medulla, the narcosis is produced by much smaller doses. Recovery is more rapid if oxygen be administered instead of air.

The phenomenon of narcosis resultant upon oxygen lack has received much attention from Verworn and his pupils, who go so far as to attribute the action of anæsthetic agents to interference with oxidation processes in the cell. The experiments performed by

Baglioni[32] and by Winterstein[33] upon the special preparation of the isolated spinal cord of the frog which Baglioni devised, show that an isolated cord which retains its excitability for forty-eight hours when kept in an atmosphere of oxygen, loses it in less than half an hour when kept in nitrogen. Baglioni further showed that by perfusion with oxygen-free saline recovery could take place up to a certain stage, beyond this saline containing oxygen was necessary. His experiments demonstrated that the narcosis was largely due to accumulated products of incomplete oxidation, which could to a great extent be washed away without previously undergoing complete oxidation.

Conclusions and discussion of results.

The similarity between the effects produced by the administration of nitrogen and by asphyxia shows that lack of oxygen is the main factor in asphyxia in the spinal animal. The results obtained further show that lack of oxygen, excess of carbon dioxide, and weak acids injected into the blood are all capable of stimulating the spinal vaso-motor centres[1]. The vaso-constrictor effect is not invariably seen in the case of carbon dioxide or injection of acids. These substances are introduced into the blood stream, and their direct action on the wall of the blood vessels being a dilator one, the pressor effect from central excitation of vaso-constrictors may be antagonised and overcome. In the case of oxygen lack, on the other hand, the production of these substances may be largely intraneuronic and they may reach a local concentration sufficiently high to excite the centres before any appreciable quantity is present in the blood stream. Apart from the direct effect of asphyxial products on the vessel wall there is a possibility that the vaso-dilator centres in the spinal cord are excited. Many observers have noted a fall of pressure on stimulation of the sensory nerves in the animal with cord transected. The frequency with which depressor responses are obtained from the cord suggests that the spinal dilator centres are readily excited. (Cf. Tigerstedt[34].)

The spinal vaso-motor centres are much less sensitive to the various agencies than the medullary centres. I have found that the medullary vaso-motor centre reacts readily to about 30 secs. oxygen lack, adminis-

[1] The term, lack of oxygen, is used to cover all the modifications in metabolic processes, and the products of such modified processes, which occur when the supply of oxygen to the tissues is inadequate.

tration of 5 p.c. CO_2, and injection of 2 c.c. $\frac{N}{20}$ lactic acid, as compared with 2 mins. oxygen lack, 30 p.c. CO_2, and 5 c.c. $\frac{N}{6}$ acid for the spinal cord. Thus the threshold of the higher centres for chemical stimuli is much lower, just as it is for nervous stimuli.

Lack of oxygen and injection of acids excite the skeletal muscle centres even up to the production of generalised spasms. Carbon dioxide appears as a rule to produce no increase in the excitability of skeletal reflexes, indeed, percentages that excite the vaso-motor centre frequently depress the skeletal reflexes. Why carbon dioxide, which excites the spinal vaso-motor centre, should not excite the spinal skeletal muscle centres, while oxygen lack and injection of acids do so, is not obvious. During the administration of carbon dioxide there is a gradually increasing concentration in the blood, which is not comparable to the sudden increase when acids are injected. In the case of nervous stimuli a gradual raising of the threshold as a result of repeated weak stimuli has been noted by Sherrington[m], and this shows some parallelism to the case of carbon dioxide, but it does not in the least explain why the spinal vaso-motor centre is excited and the skeletal motor centres are not. That carbon dioxide is capable of exciting some centres for muscular movement is shown by its action on the respiratory centre.

All the chemical agencies discussed show summation of effects. All of them produce narcosis as a result of prolonged action and for recovery from this narcosis a supply of oxygen is required. On the heart their action is practically the same, all producing marked dilatation. With the reservation regarding the action of carbon dioxide already discussed, we are led to the conclusion that since there is so much in common between the phenomena produced as the result of the action of oxygen lack, excess of carbon dioxide, and injection of acids upon the nervous mechanisms of the spinal cord, there is in all probability something in common between them as regards the manner of their action.

I am greatly indebted to Professor Starling for his advice and criticism during the course of this work.

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OBSERVATIONS ON THE SYMPATHETIC AND SACRAL AUTONOMIC SYSTEM OF THE FROG.

By J. N. LANGLEY, F.R.S., *Professor of Physiology in the University of Cambridge*, and L. A. ORBELI, M.D., *St Petersburg*.

(*From the Physiological Laboratory, Cambridge.*)

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A LARGE number of experiments have been made on the innervation in the Frog of the tissues and organs which contain unstriated muscle. When, however, a general survey is made of the results, it is found that whilst in many respects they are similar to the results obtained in the bird and mammal, in others they are different, suggesting that the plan of arrangement of the autonomic system may be different in lower and in higher vertebrates. It is obviously desirable to know whether this suggestion has any foundation in fact. In the following pages we consider the sympathetic and sacral autonomic innervation of a number of organs with the object of determining the degree of correspondence with the mammalian type[1].

[1] A brief account of some of the results given in this paper has been published in the *Proc. Physiol. Soc.* p. lxvi, 1910 (This *Journal*, XL.).

Anatomical. English Frogs (*Rana temporaria*)[1] were in nearly all cases used. In the enumeration of the nerves we follow the universal practice of experimentalists and call the hypoglossal the 1st spinal nerve. In Ecker-Wiedersheim's *Anatomie des Frosches* (ed. Gaupp) the hypoglossal nerve is called the 2nd spinal nerve. In the frog there is no spinal nerve anterior to the hypoglossal, its existence is only assumed for the purposes of comparative anatomy.

In the mammal the variations in the origin of the autonomic nerves go hand in hand with the variations in the origin of the somatic nerves. It is certain on general grounds that the same must be the case in the frog. It is known that in the frog the innervation of different regions from the spinal nerves varies considerably in different individuals. We should expect then considerable variations in autonomic innervation in different frogs even of the same species. For our purpose it is not of much importance in the upper spinal nerves to correlate the variations of autonomic and of somatic nerve origin. This is of more importance in the lower spinal nerves, and in a large number of cases after determining the autonomic innervation we have dissected the lumbo-sacral plexus, and noted the relative size of its several constituents.

We distinguish three classes of lumbo-sacral and of ischio-coccygeal plexuses[2]; depending chiefly on the origin of the recto-vesical nerve, but with which are associated differences in the relative size of the 7th to 10th spinal nerves. Since the recto-vesical nerve corresponds to the nervus erigens of mammals, now commonly called the pelvic visceral nerve or pelvic nerve, we shall usually speak of it as the pelvic nerve. The three classes are characterised as follows. (Fig. 1, cp. also Figs. 3 and 4.)

1. *Anterior Class.* The 7th nerve is about the size of the 9th. The 8th nerve is distinctly larger than the 9th. The 10th nerve is small and does not send a branch to the lumbo-sacral plexus. The pelvic nerve arises from the sciatic, or from the 9th nerve; in the former case it can, usually at any rate, be seen to arise from the 9th nerve by a little further dissection.

2. *Median Class.* The 7th nerve is distinctly smaller than the 9th. The 8th nerve is about the size of the 9th, it may be a little larger or smaller. The 10th sends a branch to the lumbo-sacral plexus. The pelvic nerve arises from the 9th nerve but receives fibres from the 10th

[1] Usually *R. arvalis*, a few apparently *R. fusca*.

[2] For an account of the observations which have been made on variations in these plexuses, we may refer to the *Anatomie des Frosches* quoted above.

either direct or by way of the plexus. Sometimes the superficial origin appears to be from the sciatic, but in this case the 8th and 9th nerves are only in apposition and can be readily separated.

Two forms may be distinguished (*a*) in which the 10th nerve is smaller than the 6th and (*b*) in which the 10th nerve is as large as or larger than the 6th, but is smaller than the 7th.

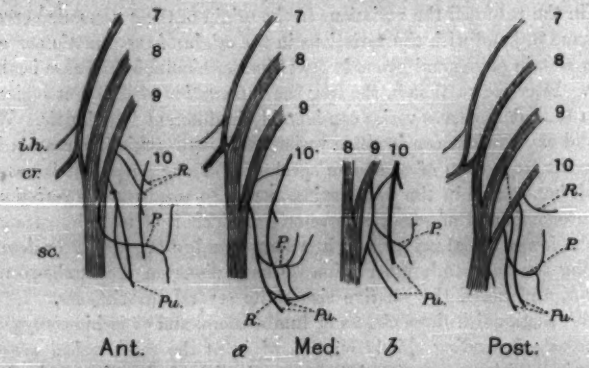

Fig. 1. Diagram of anterior, median and posterior arrangement of the lumbo-sacral and ischio-coccygeal plexuses in the frog. *P.* = pelvic nerve or recto-vesical. *Pu.* = pudic nerves. *R.* = rectal nerves. The filaments of the ischio-coccygeal plexus are usually more numerous than represented in the diagrams, and there are many minor variations with an anterior plexus. Not infrequently a branch arises from the sciatic a little below the pelvic nerve, we have not traced its peripheral distribution. Two forms of median plexus, *a* and *b*, are given.

In both anterior and median plexuses, the pelvic nerve sometimes gives a pudic branch and sometimes does not; it is for convenience only that the branch is figured in connexion with the median plexus.

In the otherwise posterior form, the dotted line indicates a filament which is present, sometimes at any rate, when the 9th nerve has a feeble effect on the bladder, it represents then the form of median plexus nearest to the posterior.

3. *Posterior Class.* The 9th nerve is distinctly larger than the 8th. The 10th nerve is as large as, or larger than, the 7th. (In two cases we found it twice as large as the 7th.) The pelvic nerve arises from the 10th nerve only.

These forms cover all the broad variations we have met with.

In general, as will be gathered from the above classification, the shifting of origin of the spinal nerves up or down affects all four of

them. But this is not always the case, especially as regards the 7th and 8th nerves on the one hand and the 9th and 10th on the other.

In the ischio-coccygeal plexus there are present besides the pelvic nerve which runs to the bladder and rectum, small nerves which run to the compressor cloacæ and external sphincter of the anus; these we may speak of as the pudic nerves since in the main they correspond to the pudic nerves of the mammal. Besides these, separate filaments not infrequently run to the rectum, possibly they are always present, for we have not paid much attention to them. In all these nerves there are numerous small variations. Thus the pelvic nerve instead of consisting at its origin of a single nerve may consist of two or three. The pudic nerves generally consist of two or three filaments, arising from the lumbo-sacral plexus (or from this and the separately running 10th nerve when the plexus is anterior) above the origin of the pelvic nerve; sometimes the main pudic arises from the pelvic nerve, this we have noticed only with an anterior or median plexus.

The direct rectal branches vary in number and position. So far as we have noticed, one or two fine nerve filaments usually arise above the pudic nerves and run to the rectum and the part of the generative ducts at the same level; some still finer filaments appear to proceed from the pudic nerves to the lower part of the rectum. This arrangement differs from that in the mammal in which all the sacral fibres for the rectum run in the pelvic nerve.

We have not paid much attention to the variations in the brachial plexus but in most cases it was formed from the 2nd and 3rd nerves (the branch from the 3rd varying in size), and in one or two cases only received a branch from the 1st spinal nerve.

The connexion of the spinal nerves from the 5th[1] downwards with the sympathetic is commonly by two or more rami, but with each nerve there is occasionally a single ramus only. For convenience we shall speak of the several rami of a nerve as its ramus when it is a question of cutting them. The 5th ganglion is either at, or more usually a little below, the level of the origin of the arteria intestinalis communis (cf. Fig. 2). We have not found any instance of the 6th sympathetic ganglion sending a branch discernible by dissection to the splanchnic nerve, such as is given in Fig. 62 of Ecker-Wiedersheim's *Anat. de Frosches*. The main branch of the splanchnic arises from, or close to, the 4th ganglion, a smaller branch arises from the 5th ganglion,

[1] We have not specially noted this point as regards the upper spinal nerves.

occasionally this ascends in the sympathetic trunk and is only discernible on teasing out under a lens.

Fine nerve filaments proceed from the sympathetic chain to the reno-genital arteries (cf. Fig. 2). They usually leave the sympathetic chain from the 5th to the 8th ganglia inclusive, occasionally there is one from the 4th ganglion to the upper reno-genital artery. Each filament consists chiefly of non-medullated fibres, but contains 1 to 4 medullated fibres (2 to 5μ in diameter), and after a short course enters the sheath of the artery. Sometimes small groups of nerve cells are present on the course of the nerve filaments.

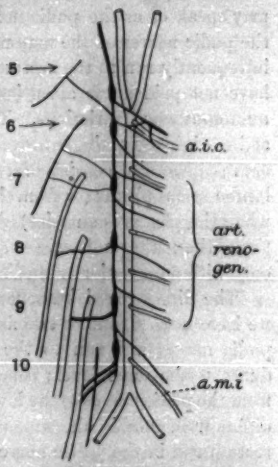

Fig. 2. Diagram of abdominal sympathetic in the frog. *a.i.c.* = art. intestinalis communis; *a.r.g.* = art. reno-genitales; *a.m.i.* = art. mesenterica inferior. The number and position of the rami communicantes varies considerably in different frogs.

The nerve filament supplying the inferior mesenteric artery is commonly too small to be certain about on dissection. On microscopic examination it can be seen running from about the 9th ganglion or a little above this, it may contain a group of 3 to 6 nerve cells. Possibly these nerve cells represent the beginning of the inferior mesenteric ganglion of higher vertebrates.

The 8th and 9th sympathetic ganglia are not infrequently continuous with one another. The 10th ganglion is fused with the 9th when the plexus is anterior. When the plexus is posterior, there are two or three small ganglia below the 9th which send off fibres to the 10th nerve and sometimes also to the 9th.

When the plexus is anterior nearly the whole of the sympathetic below the 9th ganglion runs into the 9th nerve, forming its chief or its only ramus. When the plexus is posterior the rami of the 9th nerve usually emerge from the side of the 9th ganglion, and the sympathetic below the 9th ganglion is continued on into the 10th nerve as its ramus.

The sympathetic system of *Rana esculenta* resembles that of *Rana temporaria*, and the additional size and length of the rami communicantes

make many of the experiments easier. But in the specimens we obtained, the heart stopped beating sooner than in the native *R. temp.* The lumbo-sacral plexus of *R. esc.* appears to be usually of the anterior or median type of *R. temp.*; in about eighteen specimens examined we found no case of a marked posterior plexus.

The sympathetic system of the toad (*Bufo variabilis*) is much less adapted to experiments than that of the frog, since the rami communicantes are too short for isolated stimulation, the ganglia of the sympathetic chain lying on the spinal nerves. The splanchnic nerve is however more compact and more easily isolated than that in the frog. The lumbo-sacral plexus of the toad differs from that of the frog in that the nerve fibres are more concentrated in the 8th and 9th nerves; variations in the relative number of fibres in these two nerves are less accompanied by corresponding variation in the 7th and 10th nerves.

The whole of the large intestine has commonly been called the rectum, a nomenclature which makes the whole large intestine of the frog correspond with a small part of the large intestine of the mammal. In the frog as in the mammal the large intestine is divided into three portions by the distribution of the arteries to it. The first part—$\frac{3}{4}$ths to $\frac{4}{5}$ths of the portion above the cloaca—is supplied with blood by the superior (anterior) mesenteric artery, the second part above the cloaca is supplied by the inferior (posterior) mesenteric artery. The cloaca and a little above it is supplied by the middle and inferior hæmorrhoidal arteries. The regions supplied by adjoining arteries overlap. The innervation of these regions as we shall see is different and it is convenient to have different names for them, we shall speak of them as the *upper colon* and the *lower colon* and the *rectum*; the rectum includes the cloaca and a small portion above it.

Methods. The chief difficulties in investigating the autonomic system in the frog are the shortness of the nerves, the occurrence of 'spontaneous' contractions in the unstriated muscles and the fact that an effective stimulus tends to cause rhythmic contractions which in some cases only slowly cease.

For stimulation we use the interrupted current of a du Bois Reymond induction machine; using usually in the first instance shocks that are not felt on the tip of the tongue; if these are ineffective the strength is increased, care being taken to note any signs of escape of current to the surrounding tissues. Before stimulating, excess of fluid where the nerve joins the tissue is sopped up. An interval varying in different cases from

one to several minutes is left between successive stimulations. When the general innervation had been determined the nerves having a weak effect were first stimulated in subsequent experiments.

Nearly all our observations were made under a dissecting lens. In some cases the part to be observed was exposed in the body cavity and when not under observation covered by a wad of cotton wool soaked in Ringer's fluid. In other cases the frog was placed in Ringer's fluid, in a dissecting dish having a steeply sloping side, the nerves were kept just above the level of the fluid, the viscera spread out in it.

In the frog, the pre-ganglionic fibres remain irritable for some time after the circulation has ceased, and most of the facts described in this paper can be readily seen in a frog in which the whole central nervous system has been destroyed. But in the absence of blood flow the irritability diminishes, and after a contraction recovery of irritability becomes slower and slower. In consequence as time goes on, stronger currents are required to produce an effect, and a longer interval must be left between the stimuli. Hence nerves which have only a slight action should be stimulated as soon as possible after the death of the frog.

The destruction of the central nervous system in most cases does not completely stop the circulation in the viscera. If a series of ligatures is tied in the body wall, the wall cut between them and all vessels in the way of the nerves are tied, so as to avoid loss of blood; the feeble circulation going on is retained, and this greatly facilitates continued observation.

The feebleness or cessation of the circulation is of course due to the dilation of the arteries in consequence of which little or no blood is carried to the heart. By inserting a cannula into the abdominal vein, and allowing about $\frac{3}{4}$ c.c. of Ringer's fluid to flow into it, the deficiency in the blood supplied to the heart is remedied and a good circulation results. In this method, the necessary dissection should be first carried out, all cut vessels being as far as possible tied, 15 to 20 minutes allowed for the blood to clot in any cut vessels, and the Ringer's fluid passed into the vein at a low pressure, and repeated at intervals if necessary.

The circulation is better if the brain only is destroyed, in this case the frog must be firmly pinned down after destruction of the brain, or a small dose of curari given. When any portion of the central nervous system is intact, the possibility of vascular and visceral reflexes must be borne in mind.

The tone of the blood vessels depends chiefly upon the integrity of the spinal bulb. When removing the brain without the spinal bulb we first anæsthetise the frog with 1·5 p.c. ether in water, and cut out the optic lobes and fore brain or the latter alone.

Sympathetic nerves to the iris and to the viscera.

1. *The iris.*

Budge (1855)[1], following the method then recently introduced by Cl. Bernard, sought to determine the origin of the nerves to the iris, by observing the effects of cutting them. He came to the conclusion that pupillo-dilator fibres passed from the spinal cord in the anterior roots of the 1st, 2nd and 3rd nerves. Some constriction of the pupil was caused by section of the posterior roots, but on account of its transitory nature he attributed this to a stimulation of afferent fibres.

Schipiloff (1886) adopted the same method. She inferred from her results that dilator fibres are nearly always present in the 1st nerve, rarely in the 2nd, and then only a few, and always in the 3rd. Further she considered that a few dilator fibres are present in the 4th, 5th and 6th nerves, and possibly also in the 7th. She stated that the fibres passed in all cases by the anterior roots.

Gaskell in the same year tried the stimulation method. He obtained dilation of the pupil in stimulating the sympathetic trunk below the 3rd ganglion, and inferred that the dilator fibres arose from the 4th spinal nerve.

The effect of nerve section is more open to criticism than that of stimulation, since on the one hand no effect may be produced if the number of nerve fibres cut is few, and on the other, an effect, if produced, may be due to afferent fibres, either by severing fibres running to the spinal centre, or by stimulating fibres running to the ciliary centre. But in toads used for degeneration experiments (an account of which we shall give later) we have found a marked constriction of the pupil on the side on which the roots of the 2nd nerve were cut (two cases), whilst no difference in the size of the pupils occurred after cutting on one side the roots of the 1st spinal nerve (four cases).

When stimulating the 1st, 2nd and 3rd nerves we have taken either the nerve-roots or the trunk just outside the vertebræ, with the 1st and 3rd nerves we have usually taken the latter; when stimulating the 4th

[1] References are given at the end of this paper in the order of their date.

nerve, we have taken either the trunk just outside the vertebræ, or the ramus of the nerve.

We find that the 2nd and 3rd nerves always have pupillo-dilator fibres. The effects can be obtained with very weak currents and in conditions in which an effective escape of current to the sympathetic chain is out of the question.

No effect was obtained on stimulating the 1st nerve with weak currents. With strong currents dilation of the pupil was sometimes obtained, but we attribute this to an escape to the sympathetic which in its upper part contains the fibres running from the 2nd and 3rd nerves.

From the 4th nerve also we have obtained no effect, in this case strong currents can be employed. The question of the presence of pupillo-dilator fibres in the nerves from the 4th downwards can be tested by stimulating the sympathetic trunk just above the 4th ganglion. In such experiments we have not found any effect upon the pupil.

We conclude then that, in general, pupillo-dilator fibres are confined to the 2nd and 3rd nerves. It is possible however that with a markedly posterior arrangement of nerves, the 4th may contain some fibres for the pupil. We do not think it likely that the pupillo-dilator fibres of the 2nd nerve ever get shifted up into the 1st. The anterior rootlets of the 2nd nerve are sometimes collected in two strands, and sometimes in three. On stimulation of these strands separately we have found dilation of the pupil on stimulating the lower strand, but none from stimulating the upper strand; in this case of course weak currents can only be used.

The relative effect of the 2nd and 3rd nerves has varied in different cases, usually the 2nd nerve but occasionally the 3rd had greater action, sometimes there was no appreciable difference in their effectiveness. Probably a more minute examination than we have made would show that these variations are correlated with the size of the branch sent by the 3rd nerve to the brachial plexus.

2. *The heart.*

Gaskell (1884) found that stimulation of the sympathetic a little below the point at which it joins the vagus caused marked acceleration and augmentation of the beats. In the following year the observation was followed up by Gaskell and Gadow who obtained an effect on the

heart on stimulating the sympathetic between the 2nd and 3rd ganglion, but none on stimulating between the 3rd and 4th; Gaskell (1886) concluded that the accelerator fibres arose from the 3rd nerve.

The 1st, 2nd and 3rd ganglia are so close together that there can be no certainty of isolated stimulation. We have proceeded in the manner already described for the nerves of the iris. In all our experiments nerves which caused acceleration of the heart beat also caused increased strength of the beat; indeed the increased strength was not infrequently the more obvious feature. In the following account then, acceleration means also augmentation.

The 2nd and 3rd nerves constantly caused acceleration, but in nearly all cases the effect was distinctly greater with the 3rd nerve than with the 2nd; sometimes no difference was observable with the currents employed.

The 1st nerve had no effect with weak and moderate currents; with strong currents acceleration was sometimes observed, but it was we think due to escape of current to the sympathetic.

The 4th nerve, and the sympathetic just above the 4th ganglion, not infrequently caused acceleration (in about a third of the experiments), but the effect was always less than that produced by the 3rd nerve, and generally less than that produced by the 2nd. We have in no case obtained acceleration by stimulating the ramus of the 5th nerve, or the sympathetic below the 5th ganglion.

We conclude from our results that the 3rd is the chief accelerator nerve, that the 2nd nerve constantly has accelerator fibres, and that the 4th has sometimes. The centre of oscillation appears to be in the upper part of the 3rd spinal segment, and we may infer that with an anterior arrangement of nerves, the number of fibres in the 2nd nerve is increased; with a posterior arrangement of nerves some fibres pass down into the 4th nerve.

Gaskell showed that sympathetic accelerator fibres run to the heart in the vagus, but so far as we know no experiments have been made to determine whether this is the only course. We have made eight experiments, stimulating the 3rd nerve before and after section of the vagus. In seven of these, the 3rd nerve had no effect after vagus section. In one however the accelerator action continued; the vagus contained inhibitory fibres as usual.

We may note that the condition of the heart greatly affects the degree of acceleration. If the beat is fast and strong and the circulation is good the heart may be but little influenced even by the 3rd nerve.

So far a feeble circulation is an advantage. On the other hand, there must be some circulation, or the irritability declines in the course of the experiment, and the strength of the stimuli must be increased.

3. *The œsophagus, stomach, small intestine and upper colon.*

(*a*) *Vaso-motor nerves.*

Pflüger (1855) was the first to observe that stimulation of the spinal cord caused contraction of the mesenteric arteries. Waters (1885) described the successive spinal nerves from the 3rd to the 7th as supplying vaso-motor nerves to successive, very slightly overlapping, portions of the alimentary canal. This arrangement differs from that which occurs in the mammal. In the mammal any one portion of the alimentary canal between the œsophagus and the descending colon receives vaso-motor fibres from a large number of nerves. It is, however, to be remembered that the total number of nerves is small in the frog, and that extensive overlapping in peripheral distribution might be a development of higher vertebrates. Reasons have been given by one of us for believing that in the mammal the viscera which receive their blood supply from a common artery are innervated by the same set of nerves, and that the successive branches of the abdominal aorta are innervated by successive but largely overlapping series of nerves[1]. In the frog the art. intestinalis communis supplies the lower part of the œsophagus, the stomach, the small intestine and the greater part of the large intestine. According to Waters the 3rd spinal nerve causes contraction in the vessels of the œsophagus only. If then the 3rd nerve has also a vaso-motor effect on the large intestine, his general statement of a segmental arrangement obviously breaks down. Accordingly we first made observations on the effect on the large intestine of stimulating the sympathetic below the 3rd ganglion. The result is constant and striking. The small arteries completely disappear from view and there is consequently marked pallor. These vaso-motor nerves are not readily fatigued so that the result can be obtained over and over again. Observation is favoured by the facts, that in pithed frogs, this part of the intestine is commonly quiescent, and that though on sympathetic stimulation contraction may occur, it very often does not. We have laid stress on this particular

[1] Cp. Langley, in *Ergebn. d. Physiol.* (ed. by Asher and Spiro), Jahrg. II. Abt. II. p. 848. 1903.

experiment, since it presents little difficulty and is decisive on the general question. The stimulation causes also vaso-constriction in the small intestine, in the stomach, and, as described by Waters, in the œsophagus (lower part). In the small intestine, spontaneous contractions on stimulating are more frequent than in the large intestine, so that active vaso-constriction is less readily observed, it is however never long in doubt. The œsophagus and stomach have a marked tendency to spontaneous contraction, and contract more regularly than the intestine on stimulation of the sympathetic, so that more patience is required to observe in these contraction of the vessels without contraction of the walls of the organ. It can usually be seen if the sympathetic is stimulated during relaxation of the stomach or œsophagus when the irritability of the intrinsic musculature is diminished.

The arteries of the mesentery do not respond so well as the small arteries in the wall of the gut, but these also in fresh irritable preparations close completely. We have not seen closure of the arterial trunks (art. cœliaca, art. mesen. sup., art. intest. com.) on sympathetic stimulation, but some contraction occurs in them; this is perhaps best seen when the blood-pressure is high and the pulsation considerable.

We have stimulated also the roots of the 2nd and 3rd nerves, the ramus of the 4th, the trunk and ramus of the 5th nerve, and find that each causes vaso-constriction in the whole area of the arteria intestinalis communis in the mesentery and in the alimentary canal. The effect is greater with the 3rd and 4th nerves[1] than with the 2nd and 5th.

We have rarely found any effect from the 6th nerve, in one or two early experiments slight vaso-constriction was obtained, presumably these were in extreme forms of the anterior arrangement of nerves, the actual arrangement was not however observed.

It is to be noted that whilst in a fresh preparation, stimulation of any of the above mentioned nerves causes vaso-constriction in the whole region, this ceases after a time and the contraction of the vessels is unequal in different parts of the gut. At one stimulation, one part, and at another stimulation, another part, may show very little effect or be unaffected. This marks the onset of fatigue and it comes on sooner the feebler the circulation.

Since all the small arteries in the mesentery which arise from the

[1] In two cases we found that the 3rd nerve had a greater effect than the 4th nerve on the blood vessels, but a less effect on the walls of the gut. This may have been due to some unnoticed condition of the experiments, for it is unlikely that the vaso-motor and viscero-motor fibres are different in origin.

art. intest. com. contract on stimulation of the 2nd to the 5th spinal nerves, it is fairly certain that the branches of these mesenteric arteries in the liver, pancreas and spleen receive vaso-motor fibres from the same nerves. We have not investigated this in detail, but we have seen constriction of the vessels in the pancreas on stimulating the 3rd and the 4th nerves, and contraction of the splenic artery with pallor of the spleen on stimulating the sympathetic just below the 2nd, the 3rd and the 4th ganglia, and on stimulating the ramus of the 5th nerve.

(*b*) *Viscero-motor nerves.*

Waters noticed at times contraction or peristalsis in the regions of the gut in which he observed vaso-motor effects. Steinach and Wiener (1895) described contraction or peristalsis as occurring on stimulation of the posterior, but not of the anterior roots of the 2nd to the 6th nerves. Their general scheme of innervation was much like that of Waters, but the overlapping of successive nerves was greater; they considered that each portion of the gut was innervated by two nerves. Dixon (1902) took graphic records of the contraction of the stomach, and found that, in general, stimulation of the 3rd, 4th and 5th nerves outside the spinal canal gave rise to a series of contractions and an increase of tone, the 4th nerve having the greatest effect, and the 5th usually a greater effect than the 3rd.

Observations on the movements of the alimentary canal are more troublesome than observations on the contraction of its blood vessels. When strong spontaneous contractions occur—they are nearly always stronger in the œsophagus and stomach than in the intestine—it is advisable to wait till they cease or are only slight. Since a stimulus which causes contraction generally causes a series of contractions, it is advisable to leave an interval of five or more minutes between each stimulus. It is unsafe to consider a contraction as due to a nerve stimulus, unless it is distinctly stronger than any recent spontaneous contraction, or unless it is obtained several times in an otherwise quiescent tissue: complete quiescence we must mention hardly ever occurred at the beginning of our experiments.

We find that the 2nd, 3rd, 4th and 5th nerves cause contraction in the whole region of the gut which receives its blood supply from the arteria intestinalis communis, *i.e.* the viscero-motor nerves have the same origin as the vaso-motor.

As already mentioned Waters describes the 6th nerve as causing vaso-constriction, and Steinach and Wiener describe it as causing peristalsis, in the lower part of the small intestine. We have only seen effects on stimulating the 6th nerve in two out of about twenty experiments, and they were then throughout the whole region of distribution of the art. intest. com. The occurrence or non-occurrence no doubt depends upon the arrangement of the nerves, but so far as our experience goes, it is rare in *R. temp.* for the 6th nerve to send fibres to the splanchnic, and as we have said above (p. 453) we have never seen such fibres by dissection.

We have not paid much attention to the relative degree of effect produced by the different nerves, but in many cases it was obvious that the 4th nerve was the most effective. This agrees with Dixon's result on the stomach. The relative effect of the 3rd and 5th appeared to vary, generally the 3rd but sometimes the 5th being more potent. The 2nd nerve in the few experiments in which it was compared with the 5th had a less effect than these.

The contraction is, as mentioned by Steinach and Wiener, usually in a ring, which slowly passes up or down the gut for a short distance. The peristalsis or anti-peristalsis is commonly more extensive in the stomach than in the intestine. It is rare for stimulation of one of the nerves to cause simultaneous contraction in the whole region from œsophagus to large intestine inclusive, as a rule the contraction is in one or more regions which vary with successive stimulations; but the more irritable the preparation, and the longer the interval between the stimuli, the more extensive the area of contraction. The same effect is seen on stimulating the splanchnic nerve (an experiment more easily performed in the toad than in the frog), so that the varying region in which contraction occurs is due to a varying irritability in the different parts of the gut. The varying irritability is partly at any rate due to the facts that spontaneous contractions leave a refractory period, and that they occur now in one, now in another spot. In general, contraction of the œsophagus and stomach is more often obtained, and is stronger, than contraction of the small intestine.

The primary contraction caused by sympathetic stimulation is often followed by a period of quiescence which strongly suggests the presence of inhibitory nerve fibres. We have not however specially investigated this question.

We have made a few experiments to determine whether the vaso-motor and viscero-motor fibres run by any other course than the

splanchnic. In cutting the sympathetic trunk above the 5th ganglion and stimulating the headwards end, we obtained contraction of the blood vessels and of the wall of the œsophagus, but none in the stomach. This is in harmony with the results obtained by Bottazzi (1899) and by Dixon (1902). Bottazzi observed contraction of the œsophagus of the toad on stimulating the sympathetic a little below the vagus ganglion; Dixon found no sympathetic effect on the stomach except through the splanchnic.

4. *The lower colon.*

The lower colon is supplied with vaso-motor and with viscero-motor fibres from the 6th and 7th nerves. In nearly all our experiments the 7th nerve had the greater effect, in a few the effect of the two nerves appeared to be equal. The region affected overlaps that supplied with blood by the superior hæmorrhoidal artery which branches off from the superior mesenteric artery.

We have paid more attention to the vaso-motor than to the viscero-motor effect, since the former can be seen in the small mesenteric arteries, which all show little or no spontaneous contraction, and are but little affected by spontaneous contractions of the colon. In favourable cases contraction of the inferior mesenteric artery itself is seen. Commonly, on repeated stimulation, the vaso-motor effect is alone obtained, it can then be seen that the arterial contraction is greatest in the walls of the gut, and diminishes towards the trunk of the inferior mesenteric artery.

The viscero-motor effect is on both coats of the upper colon, though the circular contraction is usually the more obvious: it is followed by anti-peristalsis often nearly reaching the small intestine.

The nerve fibres leave the sympathetic in the region of the 8th to 9th ganglia, as can be shown by stimulating the 6th and 7th nerves, after section of the trunk of the sympathetic from below upwards.

5. *The generative organs. The kidney and fat body.*

The effects of sympathetic stimulation which we have observed are contraction of the vessels of the testes, ovary, oviduct and its mesentery, fat body, kidney, and contraction of the ovary. We stimulated the spinal nerves outside the spinal cord, or their rami communicantes.

The 3rd nerve has an action on the upper reno-genital artery causing pallor of the fat body, and upper part of the reno-genital apparatus; how far down the action extends we have not determined, but we have not noticed any effect in the region supplied by the two lower reno-genital arteries.

The 4th, 5th, and 6th nerves have an action on all regions supplied by the reno-genital arteries, the 5th and 6th having a greater effect than the 4th. Sometimes the 4th has had a greater effect on the upper arteries and the 6th on the lower arteries.

The 7th nerve affects the testes, ovary and kidney, but we have not noticed any effect on the fat body. It has a greater effect on the lower than the upper arteries.

The nerve fibres leave the sympathetic in four or five small filaments which run to the arteries.

The sympathetic innervation of the *bladder* and *rectum* it will be convenient to take in connection with their innervation from the sacral autonomic system.

THE SYMPATHETIC AND SACRAL AUTONOMIC INNERVATION OF THE BLADDER AND RECTUM.

Waters (1885) described the 8th nerve as having an uncertain effect on the bladder, but as causing at times contraction of its vessels.

Gaskell (1886) found that the 7th nerve caused contraction of the circular coat of the rectum, the fibres passing to the sympathetic by the ramus communicans. He described the 8th and 9th nerves as causing contraction of the bladder and contraction of the longitudinal muscle of the rectum, the nerve fibres passing by the homologue of the nervus erigens of the mammal.

Bernheim (1892), as Gaskell earlier, obtained contraction of the bladder on stimulating the nerve running to it from the sciatic plexus; anatomically he found that the nerve arose usually from the 8th and 9th nerves, but occasionally from the 10th nerve.

The results of Steinach and Wiener (1895) were that the 6th and 7th nerve roots caused contraction of the rectum; the 7th, 8th and 9th caused contraction of the bladder, the effect being given to an approximately equal degree by the anterior and by the posterior roots.

Horton-Smith (1897) observed contraction of the rectum from the 9th and 10th nerves but not from the 6th, 7th, or 8th. He observed contraction of the bladder from the 7th, 9th and 10th nerves;

in two cases out of 15, the 7th had no effect, but the 6th caused contraction; in one case the 8th caused contraction. The 9th had sometimes no effect on the rectum, the 10th had sometimes no effect on the bladder. The effect was obtained from anterior and not from posterior roots.

Steinach (1898) confirmed the presence in the 9th nerve of motor fibres for the rectum.

Dale (1901) experimented on the frog and toad, and found as Horton-Smith had done in the frog, that the bladder receives motor fibres from the 7th, 9th and 10th spinal nerves (the 9th having the greater effect) but not from the 8th, and that the fibres left the spinal cord in the anterior and not in the posterior roots. He made a few degeneration experiments and found in these that section of the posterior roots caused no degeneration in their peripheral portions.

The bladder.

The bladder, after severance of the lower spinal nerves, is often quiescent or nearly so; and since the contraction caused by nerve stimulation, though it may be slow, is usually considerable, most of the facts with regard to the innervation of the bladder are readily determined. As with the gut, however, a stimulus which causes one contraction is apt to cause several, so that in determining the action of nerves which have a slight or a disputed effect, it is advisable to stimulate them before those known to cause obvious contraction.

Origin of nerve fibres from the spinal cord. We have made more than a dozen experiments on the nerve roots but not all the nerves were stimulated in each experiment. A much larger number were made on the nerves outside the vertebral canal; the 6th nerve has sometimes a very short course centrally of the ramus, so that not infrequently the ramus was stimulated with the trunk of the nerve; the other nerves were stimulated centrally of their junction with the ramus; the 6th and 7th nerves were usually cut peripherally of their rami before stimulation of the central portion.

We have not found any effect on the bladder on stimulating the ramus of the 5th or of any higher nerve.

The 6th nerve has not a constant effect in different frogs. In a few cases it gave fairly strong contraction, in more cases a weak one, but not infrequently no contraction was obtained by stimulating it.

The 7th nerve in nearly all cases caused contraction, usually fairly strong, sometimes weak. In the one or two cases in which no con-

traction was observed, the absence of effect may have been due to the nerve containing but a few fibres only, and to the irritability of the bladder being reduced by insufficient circulation and previous stimulation. In general, the contraction caused by the 7th nerve was weaker with an anterior than with a posterior plexus, and in the cases in which the 6th nerve produced a fairly good contraction, that produced by the 7th was weak. The 8th nerve in most cases had no effect on the bladder—a result we shall return to later.

We conclude that there is a narrow region of the cord about the 7th spinal segment from which motor fibres run to the bladder, that fibres from this region may run wholly in the 7th nerve, that a greater or less proportion of them may be shifted up and run in the 6th nerve, and that in general the shifting up is marked anatomically by the lumbo-sacral plexus being anterior in type. We have not however satisfied ourselves that there is a close correspondence between the occurrence of vesico-motor fibres in the 6th nerve and the form of the lumbo-sacral plexus. It is probable that there is some independent variation in the 6th and 7th nerve on the one hand, and the 8th, 9th, and 10th on the other.

The variation in the effect produced by the 9th and 10th nerves does however vary closely with the form of the lumbo-sacral plexus. Taking the types of plexus described earlier (p. 452), we find the effect of the 9th and 10th to be as follows.

Spinal nerve	Anterior	Median	Posterior
9	Strong contraction	Contraction	0
10	0	Contraction	Strong contraction

As a rule, with a median plexus, the larger the 10th nerve, the greater is the contraction caused by the 10th and the less that caused by the 9th nerve. Weak currents will sometimes bring out a difference not seen when stronger currents are used.

Course of the nerve-fibres from the spinal cord. Gaskell pointed out the homology of the nerve passing from the sciatic to the bladder and rectum with the nervus erigens of the mammal. He concluded that the fibres of the 8th and 9th nerves ran direct to the pelvic nerve and not by way of the sympathetic. Horton-Smith found that after section of the pelvic nerve, the 9th and 10th spinal nerves had no effect on the bladder. This result we have confirmed in half a dozen experiments[1]. The observations on the course of the vesico-motor fibres of

[1] Possibly more numerous observations would show that occasionally a few fibres run to the bladder by the direct rectal branches.

the 6th and 7th nerves are less complete. Gaskell noted that the stimulation of the ramus of the 7th nerve caused contraction of the bladder, *i.e.* that the fibres belong to the sympathetic system, and Horton-Smith added the fact that stimulation of the 7th nerve roots had no effect on the bladder after section of the ramus communicans. Gaskell on the analogy of the arrangement in the mammal considered that the further course was by the hypogastric nerves. These nerves, however, do not so far as we have seen exist in the frog. Horton-Smith made three experiments on the point, and stated that section of the pelvic nerve did not diminish the effect of stimulating the 7th nerve roots; in consequence he considered that the sympathetic vesico-motor fibres did not make their way to the bladder by way of the pelvic nerve.

In view of the anatomy of the nerves to the bladder, it is clear that the primary question to settle as to the course of the sympathetic nerve fibres is whether any of them run from the sympathetic in the rami of the 8th, 9th and 10th nerves. On this question we have made a considerable number of experiments. Since the 7th nerve nearly always has most of the sympathetic fibres for the bladder, this nerve was usually taken, it was cut at its exit from the vertebral column and peripherally of its ramus; the nerve centrally of its ramus, or the ramus itself, was stimulated. Sometimes the sympathetic trunk was cut above the 7th ganglion and stimulated below it. When the sympathetic effect on the bladder had been observed, the rami of the 8th, 9th and 10th nerves, or the nerves themselves below their rami, were cut successively in varying order, and the sympathetic stimulated after each section. One result was constant, viz. that after section of all three rami or of all three nerves, stimulation of the ramus of the 7th nerve or of the sympathetic trunk caused no homolateral contraction of the bladder. We may conclude then that all the sympathetic vesico-motor fibres which run down the sympathetic chain from the 7th ganglion leave it in the lower rami communicantes and run to the lower spinal nerves.

Having traced sympathetic fibres so far we made half a dozen experiments to determine whether the fibres left the lower spinal nerves by the pelvic nerve or by some other branches. The sympathetic fibres were stimulated in the manner given above, before and after section of the pelvic nerve (n. recto-vesicalis). In each case the section abolished the effect of sympathetic stimulation. Our experiments are not sufficiently numerous to show that the sympathetic vesico-motor fibres never leave

the lower spinal nerves by any other course than the pelvic nerve, but we think it may be safely inferred that the pelvic nerve always contains most of the sympathetic fibres.

The lower rami which it is necessary to cut in order to abolish the sympathetic effect, varied in different cases. With an anterior plexus it is usually sufficient to cut the ramus of the 9th nerve. With a median plexus, the 10th must be cut in addition. With a posterior plexus, section of the ramus of the 10th nerve alone is sufficient. We give an abstract of an experiment in which the 10th nerve contained all the sympathetic fibres; it will serve to illustrate some other facts mentioned in the text.

Exp. 1. *Rana temp.* Brain and spinal cord destroyed. Left 7th, 8th, 9th and 10th nerves tied and cut at their exit from the spinal column. Bladder observed.

Effect of nerve stimulation.

Ramus of 7th nerve on each side—good contraction of bladder.
8th nerve and 9th nerve above and below ramus—no effect.
10th nerve centrally of ramus—strong contraction.

Left side. Ramus of 10th nerve cut. After the section, stimulation of the ramus of the 7th nerve caused no contraction.

Right side. Sympathetic trunk cut below ramus of 9th nerve; after the section, stimulation of the ramus of the 7th nerve gave no contraction. The sympathetic stimulated below the point of section caused strong contraction, this no longer occurred after section of the pelvic branches of the 10th nerve. A sketch of the lumbo-sacral plexus is given in Fig. 3.

Fig. 3.

These results show that the sympathetic fibres below the 7th ganglion follow, as a rule, the course of the sacral autonomic fibres whatever that may be. We should have been inclined to regard this rule as absolute but for one experiment in which the 10th nerve was large and contained all the sacral autonomic fibres, but on the left side the sympathetic fibres as shown both by experiment and dissection ran to the 9th nerve. A sketch of the nerves on the two sides is given in Fig. 4.

It may also be mentioned that in two frogs, in both of which the lumbo-sacral plexus was anterior, section of the ramus of the 9th nerve though it greatly weakened did not abolish the effect of stimulating the ramus of the 7th nerve, the effect was abolished by subsequent section of the ramus of the 8th nerve, so that a few sympathetic vesico-motor fibres ran by way of the 8th nerve. Whether the course of the fibres in these two frogs conforms to the rule just mentioned or not

depends on whether sacral autonomic fibres were present in the 8th nerve, a point which was not determined.

Whilst, as described above, the sympathetic fibres for the bladder which are present in the sympathetic trunk below the 7th ganglion all issue from it in the lower rami communicantes, run in one or more of the lower spinal nerves, and finally in the pelvic nerve, there is another course which is occasionally, though rarely, taken by some of the sympathetic fibres, from the 6th and 7th ganglia. This is by the 6th or 7th spinal nerve to the body wall and then by the lateral ligament of the bladder, accompanying, though not necessarily closely attached to, the recto-vesical artery. Whether the 6th or the 7th nerve contains these fibres depends on which of them runs to that part of the body wall to which the lateral ligament is fixed; usually it is the 7th. When the distal part of either of these nerves causes contraction of the bladder, the effect is unaltered by isolation of the nerve up to its entrance into the body wall. In four frogs taken at random, we examined microscopically the lateral ligament on one side. In one a small nerve having a group of nerve cells on its course was found.

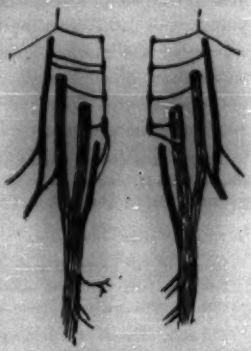

Fig. 4. *R. temp.* Right and left lumbo-sacral plexus and lower part of sympathetic. By the experimental method it was found that the sympathetic fibres for the bladder ran on the right side in the rami of the 10th nerve, and on the left side in the rami of the 9th nerve (chiefly in the lower one). The sacral autonomic fibres were confined to the 10th nerve on both sides.

The question of motor fibres for the bladder in the 8th nerve. This question is of importance in comparing the plan of the autonomic system in the frog and the mammal. In the latter the central nerves giving origin to the nerves of the hind limb do not give off autonomic fibres; there is a gap between the origin of the sympathetic and the origin of the sacral autonomic fibres. The earlier observers found no gap in the frog, Gaskell and Steinach and Wiener describe fibres as originating from the 7th, 8th and 9th nerves. On the other hand Horton-Smith, who devoted especial attention to the 8th nerve, only once in 15 cases found vesico-motor fibres in it, and Dale in some experiments on the toad found none. We have found in a considerable number of cases

that stimulation of the roots of the 8th nerve (cp. p. 475), of the nerve at its exit from the vertebral canal and more peripherally had no effect on the bladder, although contraction was obtained from one or more of the nerves above and below it. It may then be taken as certain that in *Rana temp.* there is often a gap between the origin of the sympathetic and that of the sacral autonomic nerve fibres. In such a fundamental point of structure the possibility of variation in different frogs may be put on one side, and we conclude that in the spinal cord there is always a small region at the origin of the nerves to the hind limb from which neither sympathetic nor sacral autonomic fibres are given off.

The law does not exclude the possibility of the presence of vesico-motor fibres in the 8th nerve. We have seen that there is a general relation between the type of nerve plexus and the presence of vesico-motor fibres in the various nerves forming the plexus. Hence it is not improbable that with a markedly anterior plexus, some sacral autonomic fibres normally in the 9th nerve should pass upwards into the 8th, or that with a markedly posterior plexus, some sympathetic fibres normally in the 7th nerve should pass downwards into the 8th nerve. In the latter case the gap is the 9th spinal segment.

In a certain number of the cases in which we have stimulated the 8th nerve outside the vertebral column, but centrally of its ramus we have observed contraction of the bladder though not of maximal intensity, and we are inclined to think that the effect was due to fibres issuing from the spinal cord in the nerve roots. A more decisive method of experiment is to stimulate the nerve roots; in such experiments a negative result was obtained in 13 cases, a doubtful result in one case, but as the plexus arrangement did not vary greatly from the median, this result does not settle the question whether the 8th nerve contains vesico-motor fibres with more extreme forms of nerve plexus.

Degree of contra-lateral contraction. Previous observers have shown that stimulation of the sacral autonomic nerves on one side causes contraction of the whole of the bladder, though the effect is strongest on the same side. The sympathetic has a much more unilateral effect. The extent of contraction on the opposite side varies considerably and may be confined to the median portion of the opposite side. If one pelvic nerve is cut, stimulation of the ramus of the 7th nerve gives, as we have said above, no homolateral contraction, but it may cause slight local contraction on the opposite side; we have not seen actual shortening of the bladder, but only patches of slight movement here and there. The contra-lateral effect of the sympathetic is then almost entirely due

to the crossing of nerve fibres, or outspread of stimulus, in the bladder itself, but a very trifling effect appears to be brought about by a decussation of fibres in the lower part of the sympathetic chain. It may be noted that sometimes the contraction caused by stimulation of the 7th nerve begins in the tip of the bladder and spreads to the base, suggesting a passage of fibres by the lateral arteries. Neither sympathetic nor sacral autonomic fibres have so far as we have seen any different effect on the longitudinal and circular muscle fibres.

Vaso-motor fibres. The sympathetic nerves supplying the bladder have vaso-motor as well as viscero-motor fibres. When the contraction of the bladder is prompt, this cannot be determined, since the contraction itself is sufficient to squeeze the blood out of the blood vessels. But with a weak stimulus or in partial fatigue, it can often be seen that the contraction of the blood vessels precedes the contraction of the bladder. Sometimes the 6th nerve or its ramus causes some pallor of the bladder without causing contraction of its musculature. The contraction of the blood vessels is not confined to the bladder, it occurs also in the small arteries, inclusive of those of the lateral ligament, which carry blood to it.

We have mentioned above that stimulation of the 7th or of the 6th nerve peripherally of its ramus occasionally causes contraction of the bladder. One or other of these nerves constantly causes contraction of the arteries in the body wall at the origin of the lateral ligament, it causes also more or less contraction of the arteries of the lateral ligament and this contraction may extend into the bladder vessels; in fact so far as our observations go contraction of the bladder vessels is more frequent than contraction of its musculature.

The sacral autonomic nerves do not produce so far as we have seen any change in the blood vessels that cannot be reasonably referred to a mechanical cause.

The rectum.

The rectum has a great tendency to spontaneous contraction so that observations on it are much less simple than on the bladder. We have confined our attention to one or two of the main features of the innervation.

Sacral autonomic nerve fibres. The origin of these nerve fibres from the spinal cord, and their relation to the form of the lumbo-sacral plexus, is ordinarily the same as that of the nerve fibres for the bladder. Occasionally the 10th nerve has caused contraction of the rectum

but not of the bladder as if the origin of the former were a little more posterior. The course taken by the nerve fibres is partly by the pelvic nerve and partly by other filaments given off by the 9th and 10th nerves above the origin of the pelvic nerves or by the pudic nerves. We have not however made many observations on the course of the nerve fibres in the peripheral branches. The sacral fibres cause contraction of both coats of the rectum, but the predominant effect, as is known, is the dragging down of the cloaca; the longitudinal contraction is due to special bands of muscle, corresponding functionally to the recto-coccygeal muscles of the mammal. Stimulation of the nerves is not infrequently accompanied by a brief elongation of the rectum before the contraction commences, which suggests the presence of inhibitory nerve fibres.

Sympathetic nerve fibres. On these we have not made many experiments. We are doubtful whether the special longitudinal muscles are caused to contract by sympathetic nerves though they give rise to longitudinal as well as to circular contraction. The sympathetic fibres which run to the lower colon by the inferior mesenteric artery affect the upper part of the rectum, whether they affect the lower part we have not noticed. When making experiments on the course of the sympathetic fibres to the bladder by the pelvic nerve, we made incidental observations on the rectum, and obtained some evidence that sympathetic fibres run to the rectum both in pelvic and rectal nerves; since however the observations were incidental they are not very trustworthy and require repetition.

In the cases in which the 6th or 7th nerve caused contraction of bladder by means of fibres passing in the lateral ligament we did not find any contraction of the rectum.

External sphincter. As found by Horton-Smith, the 10th nerve is usually the only nerve to cause contraction of the external sphincter of the anus. With an anterior plexus we have seen contraction of the external sphincter on stimulating the 9th nerve. The fibres run in the pudic nerves.

Viscero-motor fibres in the posterior roots.

The previous experiments on this question we have already mentioned. Briefly, Steinach and Wiener state that the œsophagus, stomach and small intestine receive sympathetic motor fibres from posterior roots only (2nd to 6th nerves) and that the large intestine and bladder receive

motor fibres in about equal proportion from posterior and anterior roots (6th to 9th nerves); whilst Horton-Smith finds motor fibres to the bladder and rectum in the anterior roots only (7th, 9th, 10th), a result confirmed by Dale for the bladder.

We have confined our observations to the nerves which offer the least chance of experimental error, viz. the 7th, 8th, 9th, the 10th and the 2nd. When stimulating the lower nerves we have confined our attention to the bladder. It is in the highest degree unlikely that what holds for the sympathetic vaso-motor and vesico-motor fibres of the 7th nerve and for the pupillo-motor and cardiac accelerator fibres of the 2nd nerve, does not hold for all sympathetic fibres.

In the lower spinal nerves we have made 5 experiments in *R. temp.* and 11 in *R. esculenta*, in two of the latter the heart stopped beating during the dissection, and the anterior root of the 9th nerve gave a weak contraction only, so that the number of satisfactory experiments in *R. esc.* was 9. The cerebral hemispheres or the whole brain was destroyed in all cases, otherwise the method of experimenting was varied; the nerve roots investigated were tied on one or on both sides; the spinal cord was sometimes left intact and sometimes removed from the 6th segment downwards; a small dose of curari was given or the frog was uncurarised, in two of the latter cases the main muscular branches of the lumbo-sacral plexus were cut on the side on which the roots were stimulated. The mode of experiment did not give any marked difference in result, but we think the best method is to destroy the cerebral hemispheres, to remove the spinal cord from the 6th segment downwards (so as to prevent the occurrence of reflex contraction of the bladder), to use uncurarised animals, and to stimulate the posterior roots before the anterior.

Steinach and Wiener consider that in uncurarised frogs, the effect of the anterior roots is much heightened by the muscular contraction acting as a mechanical stimulus. We do not think the mechanical effect can be of much importance for the following reasons. Stimulation of the peripheral end of the severed 7th nerve with a series of brief tetanising currents causes the abdominal wall to press quickly and repeatedly on the partially distended bladder, but it rarely if ever causes it to contract unless vesico-motor fibres are present in the nerve (cp. p. 470). Stimulation of the anterior root of the 8th nerve causes strong muscular contraction without—or at any rate frequently without—contraction of the bladder (cp. p. 470). As regards the 9th nerve, it is to be remembered that the threshold for somatic nerves is higher than that for autonomic

nerves, so that with a certain weak stimulation the striated, but not the unstriated, muscles contract; thus in one experiment stimulation of the anterior root of the 9th nerve with the secondary coil at 35—38 cm. caused strong tetanus of the leg, but the coil had to be pushed up to 28—30 cm. before contraction of the bladder was obtained.

Our attention was chiefly given to the 8th and 9th nerves, but in six experiments we obtained more or less repeatedly contraction of the bladder on stimulating the anterior roots of the 7th nerve, and none from stimulating the posterior roots.

In each of the 14 experiments the posterior roots of the 8th nerve had no effect; the anterior roots had no effect in 13 cases, and a doubtful effect in one case.

In 13 of the experiments, the posterior root of the 9th nerve had no effect until the current was made so strong that there was obvious escape to the anterior root. On the other hand the anterior root gave a good contraction with currents much too weak to be felt on the tongue or lip. Thus usually contraction was obtained when the secondary coil was 25 to 30 cm. from the primary, the current being first felt with the coil at about 17 cm. (cp. also Exp. 2 infra).

In 2 experiments (*R. esc.*) the roots of the 10th nerve were stimulated; no effect was obtained from the posterior root; contraction of the rectum but not of the bladder was obtained from the anterior root.

It must be mentioned that in several of these cases there were some spontaneous contractions of the bladder, in these we waited until the contractions were slight and only occurred at intervals of not less than three minutes before making decisive observations. No reliance can be placed on ocular observation when the spontaneous contractions are frequent unless the contraction produced by nerve stimulation is much stronger than that occurring spontaneously. It may be thought that in these cases the irritability of the bladder would be reduced so that motor fibres if present in the posterior roots would no longer produce an effect. It is probable that some diminution of irritability occurs, but if it does, it is insufficient to cause any appreciable difference in the effect of stimulating the anterior roots.

In two cases the bladder was observed for two days after section of the nerve roots at a time when it was completely quiescent; nothing could be more striking than its immobility on stimulating the combined roots of the 8th nerve or the posterior root of the 9th nerve, and its prompt contraction on stimulating the anterior root of the 9th nerve. The protocol of one of these experiments is given (Exp. 2), it illustrates

also the continued high irritability of the anterior root of the 9th nerve after section.

Exp. 2. *Rana temp.* Brain destroyed. Cord cut at 6th spinal segment and part below removed. Roots of the 8th nerve tied together. Roots of the 9th nerve tied separately on each side. Both sciatic nerves cut in the thigh. Right side of the bladder exposed; the frog and the severed abdominal muscle fixed by pins.

Spontaneous strong contractions of the rectum and weaker contractions of the bladder, the relative strength of the contraction of the two organs varying. After 45 mins. the spontaneous contractions were trivial.

Tetanising currents were used for stimulating the nerve roots. With the secondary coil at 15 cm. the currents were doubtfully felt on the tongue. Nerves stimulated on the right side.

3.50. Stimulate both roots of 8th nerve for 30 secs.; sec. coil at 8 cm.—no contraction of bladder or rectum.

3.52. Stimulate posterior root of 9th nerve for 30 secs.; sec. coil at 8 cm.—no contraction of bladder or rectum.

3.54. Stimulate anterior root of 9th nerve for 5 secs.; sec. coil at 30 cm.—in 2 secs. good contraction of the bladder.

3.56. Stimulate anterior root of 9th nerve for 5 secs.; sec. coil at 15 cm.—at once good contraction of the bladder, and in 3 secs. strong drawing down of rectum.

The observations were repeated and gave constant results. A day later, the bladder being quiescent, contraction of the right side, though slow and rather weak, was still obtained by stimulating the anterior root of the right 9th nerve, the secondary coil being at 28 cm.

Two days after the nerve section, the left side of the bladder was exposed; there was no spontaneous contraction during 15 minutes; the roots on the left side were stimulated, those of the 8th nerve and the posterior root of the 9th, the secondary coil varying from 30 to 8 cm., did not cause a trace of contraction in the bladder, whilst the anterior root of the 9th nerve, the secondary coil being at 30 cm., caused at once good contraction. The 8th nerve roots and the anterior root of the 9th caused, as throughout, contraction of the striated muscles supplied by the uncut branches.

In one experiment we obtained contraction of the bladder on stimulating the posterior root of the 9th nerve. There was a tendency to spontaneous contraction of the bladder in this case, but on protracted observation it seemed clear that the result was genuine. The contraction however was very much weaker than that caused by stimulating the anterior root. A weak tetanising current applied to the posterior root produced no effect. Contraction was first produced with the secondary coil at 12 to 10 cm., at this distance the shocks were distinctly felt on the tongue, but in no way painfully. Though the requisite current was not unusually strong for nerve stimulation, it was very much stronger than that required to cause contraction when the anterior root was stimulated. The latter caused contraction when the secondary coil was at 20—25 cm. With autonomic nerve stimulation it is common to find

that when few fibres are present, a stronger stimulus must be applied to produce an effect than when there are many fibres. We conclude then that in this case, some vesico-motor fibres were present in the posterior roots, but very few relatively to those in the anterior root.

In this connection it is worth mention that Steinach and Wiener (cp. Steinach, 1898) found rather strong currents to be necessary to produce an effect from the posterior roots. Steinach assumes that it is a characteristic of vaso-motor and viscero-motor fibres to require a strong stimulus. This is not the case either in the frog or in the mammal. All effects of cranial autonomic, sympathetic or sacral autonomic nerves can be produced by shocks which are not perceptible or barely perceptible to the tongue, except when the number of fibres stimulated is few. It is true that the threshold is higher than for somatic nerves, but very weak shocks are in both cases effective.

Our other experiments we may mention briefly. The roots of the 2nd spinal nerve were stimulated in three experiments, the posterior root had no effect, the anterior (consisting of 2 or 3 strands) caused dilation of the pupil. Budge, as we have mentioned above (p. 457), by the method of nerve section, arrived at the conclusion that the dilator fibres for the pupil ran in the anterior and not in the posterior roots.

The results given in this section may be summed up as follows:

Nine nerve roots containing sympathetic nerve fibres were stimulated in 9 frogs, the posterior roots in no case gave any effect, the anterior roots gave an effect in each case.

Fifteen nerve roots containing sacral autonomic nerve fibres were stimulated in 13 frogs, the posterior root in one case had a slight effect, the anterior root in all cases had a strong effect.

The nerve roots of the 8th nerve were stimulated in 14 frogs, and no effect was obtained from the posterior roots. The anterior roots gave no effect in 13 cases, the effect was doubtful in one case. The lumbo-sacral plexus did not vary greatly from the median form in any of the experiments.

Whilst then we so far agree with Steinach and Wiener that we hold there may be autonomic fibres in the posterior roots, we do not agree with him in regarding this as a normal occurrence. We conclude from the results obtained by Horton-Smith, Dale and ourselves, that the presence of autonomic fibres in the posterior roots is a rare phenomenon, on a par with the occasional occurrence of somatic motor fibres in these roots, as found by Horton-Smith and confirmed by Wana[1] and by Dale.

[1] Wana (*Pflüger's Arch.* LXXI. p. 555. 1898) found somatic motor fibres in the posterior roots in a much smaller percentage of cases than did Horton-Smith.

Summary of the chief results.

The origin from the spinal nerves of the sympathetic nerve fibres to the organs we have investigated is as follows:—

TABLE I.

Organs:—	Iris	Heart	Œsophagus to upper colon[1] inclusive. Pancreas, spleen	Generative organs. Kidney. Fat body	Lower colon	Bladder
Arteries:—			Arteria intestinalis communis	Arteria reno-genitalis	Arteria mesenterica inferior	
Spinal Nerves	**2**	2	2	⋮	⋮	⋮
	3	**3**	**3**	*3*	⋮	⋮
		4	4	4	⋮	⋮
		⋯	5	**5**	⋮	⋮
				6	*6*	**6**
				7	**7**	**7**

Black type = nerves having usually the maximal effect in the series.
Italics = ,, ,, a weak effect.
Dotted line = ,, frequently having no effect.

Notes:—Iris. The 3rd nerve has occasionally a greater effect than the 2nd.
Heart. Occasionally the 2nd nerve has as great an effect as the 3rd.
Art. int. com. In rare cases the 6th has a slight effect, we have found it so seldom that we omit it from the Table.
Art. ren. gen. The 3rd nerve runs to the upper arteries, the 7th chiefly to the lower, and occasionally the maximum effect of the 6th is lower than that of the 4th.

No sympathetic fibres issue from the spinal cord in the 1st spinal nerve (hypoglossal).

The origin of the nerve fibres varies somewhat in different frogs, this variation probably coinciding with a similar variation in the origin of the somatic nerves.

The sympathetic nerves produce easily observed vaso-motor effects, the contraction is greatest in the final arterial branches and decreases towards the arterial trunks. They produce also less easily observed viscero-motor effects on both coats of the gut of the ovary.

Each artery given off by the abdominal aorta receives nerve fibres from more than one spinal nerve. Each nerve which sends nerve fibres to an artery supplies the whole region of the distribution of the artery, thus the 2nd, 3rd, 4th, 5th spinal nerves send fibres to the whole region supplied by the arteria intestinalis communis.

[1] We divide the large intestine in accordance with its arterial supply into upper colon, lower colon and rectum.

Sympathetic vaso-motor nerves pass to the œsophagus but not, so far as we have seen, to the stomach, by way of the vagus.

As a rule the only course taken by the sympathetic fibres from the sympathetic trunk to the bladder is by way of the rami communicantes of those spinal nerves which have sacral autonomic fibres (cf. below); their further course is by the pelvic nerve. Occasionally, some vesico-motor and vaso-motor fibres run outwards in the 7th, or more rarely the 6th nerve, to the body wall, and ther. in the lateral ligament of the bladder.

The origin of the sacral autonomic fibres for the bladder and rectum is in most cases in obvious correspondence with the relative size of the lower spinal nerves. We distinguish anatomically an anterior, a median and a posterior arrangement. In the anterior class, the sacral autonomic fibres are in the 9th nerve, in the median class they are in the 9th and 10th, in the posterior class they are in the 10th nerve. Occasionally the origin of the fibres to the rectum appeared to be a little more posterior than that of the fibres to the bladder.

The 8th spinal nerve as a rule does not convey from the spinal cord either sacral autonomic or sympathetic fibres, a result already obtained by Horton-Smith and by Dale. We have in some cases obtained evidence of the presence of vesical nerve fibres in the 8th nerve, and we think it probable that in extreme forms of the lumbo-sacral plexus there is a shifting up of sacral autonomic fibres or a shifting down of sympathetic fibres into this nerve.

The sacral autonomic fibres for the bladder and the sympathetic fibres for the iris, heart and bladder leave the cord in the anterior roots, and only rarely do any run in the posterior roots; this result is in general correspondence with those of Horton-Smith and of Dale.

Comparison of the autonomic system with that of the mammal[1].

The results given in the preceding section afford a basis for comparing the sympathetic system of the frog with that of the mammal. We see that the serial order in which the nerve fibres leave the spinal

[1] Cf. Langley. *Text-Book of Physiology*, ed. by Schäfer (Edinburgh), II. p. 616. 1900; *Ergeb. d. Physiol*, ed. by Asher and Spiro, II. Abt. 2, p. 819. 1903, for autonomic system of mammal, and This *Journal*, XXX. p. 221. 1903, for that of the bird.

cord is in the main the same. In both frog and mammal the origin of the nerve fibres for the iris is a little anterior to that of the fibres for the heart.

The origin of the nerve fibres to the abdominal and pelvic viscera goes hand in hand with their arterial supply. The arteria intestinalis communis in the frog receives nerve fibres from a series of nerves and each of these nerves sends fibres to all the organs to which the artery runs. Broadly, the same holds for the mammal, but there is evidence of more differentiation. In the mammal instead of an art. intest. com. dividing into two main branches the cœliac and the superior mesenteric, the cœliac and superior mesenteric arteries arise separately from the abdominal aorta, the maximal effect on the organs supplied by the former is from nerves higher in the series than those which give the maximum effect on the organs supplied by the latter[1], and it is doubtful whether the highest of the series supplies the superior mesenteric artery or whether the lowest of the series supplies the cœliac artery.

The renal arteries as in the mammal are supplied by a series of nerves which largely overlap those supplying the cœliac and superior mesenteric arteries. The details of arrangement are here different in the frog and mammal in correspondence with the anatomical differences of the kidney in the two cases. In the frog, four or five arteries are given off from the abdominal aorta to the reno-genital apparatus; the maximal effect on the upper arteries is obtained from a higher series of nerves than that which gives a maximal effect on the lower arteries, and so far as we have seen the uppermost nerve does not run to the lowest artery, or the lowest nerve to the highest artery.

Following the reno-genital arteries is the inferior mesenteric artery; here the arrangement of the nerves is similar to that in the mammal except that the overlapping in the origin of the nerves with those supplying the superior mesenteric artery is less; this is probably connected with the fact that in the frog several renal arteries intervene between the superior and inferior mesenteric artery.

The extent of the region of origin of the sympathetic in relation to that of the limb plexuses is much as in the mammal, but in the frog it extends rather farther into the region of origin of the brachial plexus.

It may be noticed that sympathetic vaso-motor fibres for the bladder are better developed in the frog than in the dog, cat or rabbit.

[1] Although we have not been able to satisfy ourselves that this is the case in the frog, we have obtained some indications that it does.

Further the results given above enable us to compare in the frog and mammal not only the sympathetic system, but some of the main features of the whole autonomic system.

From the results of previous observations we know that the cranial autonomic system is made up of a mid-brain portion sending fibres to the ciliary ganglion; a bulbar portion consisting of fibres from the facial (homologous with the n. interm. of Wrisberg) with scattered peripheral ganglia, and of fibres from the vaso-glossopharyngeal nerves also with peripheral ganglia. We have seen that the 1st spinal nerve segment, and the 8th spinal nerve segment, or a portion of the cord close to it, contains no autonomic nerve fibres. Thus the central nervous system, as in the mammal, sends out autonomic fibres from four separate regions, the tectal region supplying the eye; the bulbar region supplying the anterior part of the alimentary canal and the structures connected with it; the mid-spinal region (sympathetic) supplying all the structures; and the sacral region supplying the posterior part of the alimentary canal and the structures connected with it.

The autonomic fibres as in the mammal and bird normally leave the spinal cord in the anterior and not in the posterior roots, but occasionally a few fibres may be present in the posterior roots, a condition described as occasional in the bird but very doubtful as ever occurring in the mammal.

In the course of the sympathetic fibres from the main chain, there is a difference from the mammal, which is interesting as indicating a more primitive form. In the mammal whilst a considerable portion of the sympathetic fibres which run to the regions supplied by cranial autonomic nerves run with these nerves nearly the whole of their course, some—the sympathetic fibres to the thoracic viscera—take a short cut and join the cranial autonomic fibres near their periphery. Where the peripheral distribution of the sympathetic fibres is the same as that of sacral autonomic fibres, nearly the whole of the former take a short cut and run in the hypogastric nerves. In the frog, neither of these short cuts is taken. The fibres to the thoracic viscera (as shown by Gaskell in the case of the accelerators of the heart) run in the trunk of the vagus, and the fibres to the bladder run in the lumbo-sacral nerves.

We conclude then, that whilst there are minor differences, the general plan of arrangement of the autonomic system for the structures here dealt with is the same in the frog as in the mammal and the bird.

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THE ORIGIN AND COURSE OF THE VASO-MOTOR FIBRES OF THE FROG'S FOOT[1].

By J. N. LANGLEY, Sc.D., F.R.S., *Professor of Physiology in the University of Cambridge.*

(*From the Physiological Laboratory, Cambridge.*)

Method. The observations unless otherwise mentioned were on *Rana temp.*, the circulation in the web being watched under the microscope. The cerebral hemispheres were destroyed or excised in all cases. In nearly all cases a portion of the rest of the central nervous system was also destroyed or excised. Thus in different experiments, the intact part began at the optic lobes, at the spinal bulb, midway in the spinal bulb, at the top of the spinal cord, below the brachial nerves and just above the lumbar nerves. Sometimes the whole of the central nervous system was destroyed. When a part was excised, the frog was first etherised. When nerves were stimulated which caused muscular contraction, a small dose of curari was nearly always given.

When after an operation the circulation is poor, the preparation may be placed aside for an hour or two, either in a dish containing water to a depth of 1 cm. (curarised preparation), or covered with blotting paper soaked in Ringer's fluid and protected from evaporation (non-curarised preparation). The circulation improves partly by the absorption of water, and partly by some recovery of arterial tone. A better plan is to tie a cannula into the abdominal vein and to inject about $\frac{1}{2}$ c.c. of Ringer's fluid as required.

If the nerves are to be stimulated on successive days, it is better to remove than to destroy the brain or other portion of the central nervous system; after destruction there appears to be some absorption of products causing an earlier cessation of the beat of the heart. In one case, I removed the brain under ether, gave a small dose of curari,

[1] A summary of some of the results given in this Paper has been published in the *Proc. Physiol. Soc.* p. lxiii. 1910. (This *Journal*, XL.)

and in the course of the next 24 hours, removed in two operations the whole of the spinal cord; for a day after the total removal, the circulation remained rapid.

In determining the spinal nerves by which vaso-motor fibres leave the spinal cord, it is not necessary to stimulate the nerve-roots unless it be supposed that such fibres arise from the spinal ganglia and have no connection with the spinal cord. The best procedure to adopt varies with the different nerves: for the 2nd, 8th and 9th nerves it is best to take the nerve-roots; for the 1st, 3rd, 4th, 5th, 6th and 7th nerves it is best to ligature and cut the nerve just outside the vertebral canal, to cut the nerve past the ramus communicans[1] and to stimulate it immediately below the ligature. The nerves outside the vertebral canal can be exposed either from the dorsal or the ventral surface, the former interferes much less with the circulation.

Whatever method was employed, similar results were obtained provided proper precautions were taken.

Precautions. There are certain conditions in which stimulation of vaso-motor nerves may either lead to doubtful results or to no result at all.

(*a*) *The occurrence of spontaneous contractions in the arteries of the web.* Spontaneous rhythmic contractions, first noticed by Saviotti, have been described by most observers. As previous observers, I have found that spontaneous contractions depend chiefly upon the central nervous system, but that they may occur after its destruction and after section of the sciatic nerve; that the contraction is often unequal in different arteries of the web, and even in different parts of one artery, one part of an artery contracting little or not at all, another part contracting considerably or completely. Commonly, stimulation of the vaso-motor nerves which run to the web increases the spontaneous contractions; and not infrequently, if there are no such contractions, stimulation causes a short series of them. I made some observations one or two days after section of the sciatic nerve, in these the spontaneous contractions were slight or absent. They were also slight or absent after a large dose of curari.

(*b*) *Reflex contraction.* The occurrence of reflex contraction in the vessels of the web has been shown by Huizinga and others.

There can be little doubt that normally slight sensory stimuli applied to the skin cause quick contraction in the vessels of the web,

[1] For convenience I use the term "ramus," whether one only is present, or whether there are two or more.

which may lead to complete cessation of the circulation lasting some time after the stimulus has ceased. I have made a few observations on frogs with the fore-brain destroyed and curarised. On taking the body between finger and thumb and lightly pressing once or twice, the arteries contracted and the circulation stopped. Cold water applied to the skin had a like effect, and with diminished irritability appeared to be a more effective stimulus. According to Huizinga (1875), pinching the toes causes dilation, but pinching the skin elsewhere causes contraction. In my experiments, the effect of lightly touching or of pinching varied. From the toes the result was usually dilation, or after-dilation, but sometimes there was primary contraction. From the skin of the rest of the body the result was usually contraction followed by dilation. When the reflex action is slight it may shew itself only in the artery becoming moniliform. The dilation is no doubt mainly passive (cf. p. 493).

(*c*) *Fatigue.* It is important not to stimulate the nerves at too short intervals for although in some cases contraction of the arteries can be obtained repeatedly when the stimulus is applied as soon as the vessels have dilated, yet in other cases the fatigue is rapid. The worse the circulation, the more rapid naturally is the fatigue.

(*d*) *Inequality of response in different arteries.* When a nerve has but a few vaso-motor fibres, stimulation of it never, so far as I have seen, causes equal contraction in all the arteries of the web; commonly one or two contract well and others not at all. In order then to be certain that a nerve has no effect, all the arteries must be examined. Even when a nerve has a good and widespread vaso-motor effect the arteries do not usually contract equally either in rate or in extent. It has been mentioned above that spontaneous contractions are commonly unequal in different arteries, and that a contraction tends to lower irritability for a time; probably then spontaneous contractions tend to cause inequality of contraction on nerve stimulation.

(*e*) *Action of curari.* When stimulating the 8th and 9th nerves it is almost essential to give curari in sufficient amount to paralyse the skeletal muscles. But curari must be used with care; in proportion to its amount, and up to a certain limit in proportion to the duration of its action, it weakens the vaso-motor nerves. Thus an hour or two after a large dose, or on the next day after a somewhat smaller dose, nerves normally active give little or no effect. Moreover curari in amount beyond that required to paralyse the skeletal muscles, alters the condition and fatigue rapidly sets in.

The origin of the vaso-motor nerves for the foot.

Wharton Jones (1853) found no dilation of the vessels of the web on removal of the part of the spinal cord containing the origin of the nerves to the leg (the 7th, 8th and 9th), although dilation followed section of the sciatic nerve. He concluded that the vaso-motor nerves arose from the sympathetic and not from the spinal cord.

Lothar Meyer (1854) observed dilation of the vessels of the web on cutting the connection of the sciatic with the sympathetic.

Pflüger (1855) found constriction of the vessels of the web on simultaneous stimulation of the anterior roots of the nerves giving origin to the sciatic plexus, *i.e.* of the anterior roots of the 7th, 8th and 9th nerves.

Gunning (1857) arrived at results opposed to those of Meyer. He observed no dilation on cutting the rami communicantes of the sciatic, but found it to occur on section of the nerves near their exit from the vertebræ.

Lister (1858) showed that stimulation of the central nervous system caused contraction of the blood-vessels, and he considered that the nerve fibres arose from the same region of the cord "as do those through which sensation and motion are effected in the hind legs." Some of his experiments however indicate that the origin is not confined to this region.

Nussbaum (1875) observed that the stimulation of afferent nerves caused contraction of the arteries after destruction of the brain, spinal bulb, and upper portion of the cord, but not after destruction of the whole central nervous system. He found also that section of the roots of the 8th and 9th nerves caused no dilation of the vessels, but that this occurred when the 7th nerve was also cut. After section of the three nerves he did not obtain reflex contraction from sensory stimulation.

Huizinga (1875) obtained reflex contraction of the vessels of the web by pinching the fore-limbs after the cord below the origin of the brachial plexus had been destroyed; and he concluded that the origin of the nerves was not confined to the lumbar region.

Jegorow (1892) obtained contraction of the blood-vessels by stimulating the rami communicantes of the 4th and 5th spinal nerves as well as those of the sciatic plexus. He considered however that the vaso-motor nerves arose from the sympathetic and had no connection with the spinal cord, amongst other reasons because he found no contra-lateral

reflex contraction in stimulating the central end of the crural or sciatic nerves.

In the preceding and in my own observation, the hypoglossal is reckoned as the 1st spinal nerve.

Several of the observations quoted above, especially those of Lister and Nussbaum, make it certain that the central nervous system governs the vaso-motor nerves, in the frog as in the mammal, and that the results tending to show its sole dependence upon the sympathetic system were based upon imperfect experiments. This conclusion is easily confirmed. In a weakly curarised frog, stimulation of the optic lobes, after removal of the cerebral hemispheres, or of the top of the spinal cord after removal of the whole of the brain and spinal bulb, causes a more or less complete contraction of the arteries of the web; the circulation often ceasing for two or three minutes.

The previous observations do not however settle the question of the nerves by which the vaso-motor fibres leave the spinal cord.

Whilst all observers who hold that there are vaso-motor fibres in the nerve-roots consider that they are present in the 7th, 8th and 9th, Huizinga gives them also a higher origin. Some of the experiments given by Lister, and some of those given by Jegorow, are in favour of the presence of vaso-motor fibres from the higher nerves, though the former does not draw this conclusion, and the latter denies that the fibres come from the spinal cord at all. But no observations, so far as I know, have been made on the effect of stimulating the nerve-roots, with the exception of those made by Pflüger, and in his experiments the 7th, 8th and 9th nerves were not stimulated separately.

I have made six experiments on the lower nerve-roots, one being on *Rana esculenta*. The anterior and posterior roots were stimulated separately in two of the experiments. In none did the roots of the 8th or 9th nerves cause contraction of the vessels of the foot[1].

The 7th nerve had a distinct vaso-motor action in all cases but one, and in this case a few vaso-motor fibres may have been present, since curari had been given in more than the usual amount. The rapidity of contraction in some arteries and its extent varied considerably in different experiments, and this may reasonably be attributed in the main to a little shifting up or down of the origin of the vaso-motor

[1] Contraction of the vessels may be obtained by stimulating with moderately strong currents the 8th nerve at its exit, but in this case there is escape of current and probably the ramus of the 7th nerve which runs across the 8th is stimulated. More or less cessation of the circulation is caused by stimulating the 8th or 9th nerves if curari is not given; this is a mechanical effect of the muscular contraction.

fibres. How far this shifting was correlated with shifting of the fibres of the lumbo-sacral plexus was not determined since the plexus was not dissected in a sufficient number of cases. Some dissections were however made, and using the terms anterior, median and posterior as defined by Orbeli and myself[1], the 7th was found to have vaso-motor fibres both with a moderately anterior and with a moderately posterior form of plexus. In the case in which the 7th nerve had no effect, the plexus was markedly anterior in so far as the 7th nerve was nearly as large as the 8th nerve[2].

Two or three experiments only were made with each of the nerve-roots from the 6th to 3rd (the anterior roots alone being sometimes taken) since these nerve-roots are easily injured, and experiments practically as conclusive can be made on the nerve-trunks or on their rami communicantes. Good contraction of one or more arteries in the web was obtained by stimulating each nerve, but commonly the effect ceased after a few stimulations, and was then only obtained by placing the electrodes on the intervertebral canal. The 2nd nerve-roots gave in three cases no contraction that I was able to observe. In one case the absence of effect was less certain, but after repeated stimulation I came to the conclusion that no vaso-motor fibres were present.

No vaso-motor nerves for the web were found in the 1st nerve (3 experiments) nor in the 10th nerve (2 experiments)[3].

The effects of stimulating the 4th to the 7th nerves can be better observed by taking them outside the spinal canal (cf. above, under *Method*), and those of the 3rd nerve by taking the trunk of the sympathetic below the 3rd sympathetic ganglion. Observations can be carried on for hours, and often on successive days. On stimulating each of these nerves, I have seen complete closing of one or more arteries of the web. With the 3rd and 4th nerves the contraction was usually, and perhaps always, local; this was sometimes the case with the other nerves, and the contraction was always unequal in different arteries. In one case the sympathetic below the 3rd nerve caused repeatedly contraction in one only of two adjoining arteries in the 3rd web. The 4th nerve caused good contraction in the same artery and a little in the adjoining one. The effect increased with each nerve from the 4th to the 7th. On turning again to the 3rd nerve, the effect was as before, but a slight contraction was obtained in the

[1] Langley and Orbeli, this *Journal*, XLI. p. 452. 1910.

[2] The shift upward had not much affected the 9th nerve since this was only slightly smaller than the 8th. The 10th nerve was small.

[3] In one of these the lumbo-sacral plexus was anterior, in the other median (*b*).

adjoining artery. In each case the effect was obtained with a current not felt on the tongue. In general the effects have increased in passing from the 3rd nerve downwards, but in two experiments the 6th had a greater effect than the 7th. It must be mentioned that sometimes there is distinct contraction without complete closure of the artery.

The 8th and 9th nerves can be stimulated centrally of their rami, but this method is less satisfactory than that of stimulating the nerve-roots, since if strong currents are used, there is considerable risk of an outspread of the current to the part of the nerve where the ramus joins it. There is however little difficulty in finding a strength of current which causes no vascular contraction when applied to the part of the 8th or 9th nerve near its exit from the spinal canal, but causes contraction when similarly applied to the upper nerves.

The 10th nerve has a longer course centrally of its ramus (posterior plexus); stimulation of it in my experiments had no effect on the circulation in the web.

Course of the vaso-motor fibres.

The preceding experiments have shown that the vaso-motor fibres for the web ordinarily pass from the spinal cord in the 3rd to the 7th spinal nerves inclusive, and that most of them at any rate leave the nerves by the rami communicantes and run to the sympathetic chain. The anatomical connection of the 3rd—6th nerves makes it certain that all their vaso-motor fibres for the web, run to the sympathetic by way of the rami communicantes. As regards the 7th nerve, section of its ramus prevents stimulation of its roots from causing any contraction in the vessels of the web, and no contraction is produced by stimulating the 7th nerve peripherally of its ramus (cf. however p. 493).

From the sympathetic chain, the vaso-motor fibres might proceed either by way of the rami communicantes of the nerves which send fibres to the sciatic nerve, or by way of the arteries.

That some vaso-motor fibres run in the sciatic nerve to the web is past question. It has been shown either by noting enlargement of vessels after section of the nerve, or, by noting contraction of the arteries, and slowing or cessation of the circulation on stimulation of the peripheral end of the nerve, by Wharton Jones, Pflüger, Lister, Putzeys and Tarchanow, Nussbaum, Huizinga, Ellis, and Arkharoff. It is true that Dogiel failed to observe increased blood-flow on cutting the sympathetic, and that Humilewski and Jegorow, working in Dogiel's laboratory, failed to observe contraction of the

blood-vessels on stimulating it, but their negative results have no weight in view of the numerous positive ones. I have stimulated the peripheral end of the sciatic at various times in the last thirty years and in about twenty frogs in the course of these experiments after movement of the limb had been prevented by a small dose of curari, and in all have observed, under the microscope, contraction of the arteries and more or less widespread cessation of circulation in the web. If sufficient time be left between the stimuli, and the amount of curari given be not too great, the effect can be observed over and over again. In a fresh irritable preparation, the contraction ordinarily begins a second or two after the beginning of the stimulus; and the small arteries close two or three seconds later, and the contraction may last a minute or more after the end of the stimulation. When the irritability is lessened, it may require 25 to 30 seconds' stimulation before the maximum contraction is attained, and not all the arteries may be completely occluded. Some details of the effect of stimulation will be given later (p. 495). The contraction of the arteries can be seen when there is no circulation, provided this has not lasted too long. If the brain of a frog is destroyed, curari given to paralyse the somatic motor nerves, and then the spinal cord destroyed, there is usually no circulation of blood in the web; but on stimulating the peripheral end of the cut sciatic, the stagnating blood (after a primary flow onwards, cf. p. 493) is seen to be driven back in the small arteries, and onwards in the veins till the small arteries become empty or nearly so. The duration of the irritability of the vaso-motor nerves of the sciatic after the circulation has ceased varies greatly in different cases; and depends on whether and how much the sciatic is stimulated.

The question which remains is whether any vaso-constrictor fibres pass to the web by way of the artery as well as by the sciatic. This question was answered in the negative by Huizinga (1875), who found that after section of the sciatic, no contraction could be obtained reflexly in the web of the cut side. Jegorow (1892) on the other hand states that section of the sciatic in the thigh makes no difference to the effect of stimulating the sympathetic, and he concludes that the vaso-motor fibres run by some other course than the sciatic and probably by the blood-vessels. I have experimented in several ways:

(*a*) The reflex method employed by Huizinga. The sciatic was cut on one side and the skin lightly touched or pinched: contraction was only obtained on the intact side.

(*b*) The method employed by Jegorow. A loose thread was placed under the sciatic in the middle of the thigh, the sympathetic trunk at

various points from the 3rd to the 6th ganglion was stimulated, and the usual contraction of the arteries observed. The sciatic was then cut, and after the contraction caused by the section had passed off, the sympathetic was again stimulated; the stimulation caused no trace of vaso-constriction, nor did it at any later time. Sometimes there was an increase in rate of circulation due to contraction in other regions and the resulting rise of blood-pressure (cf. p. 493).

(c) The sciatic was cut on one side and each web placed under a microscope. The optic lobes or upper part of the spinal cord were exposed and stimulated; the part of the central nervous system above this having been removed under ether. There were contraction and cessation of circulation on the side with the intact sciatic, whilst the circulation continued in full course on the side on which the sciatic had been cut. In one case the occurrence of spontaneous contractions, strong on the uncut side, interfered with the course of the experiment, but in three other cases the spontaneous contractions were slight, and stimuli applied at intervals of 8 to 10 minutes gave perfectly constant and striking results.

Notwithstanding, then, that Jegorow was fully aware of the occasional occurrence of strong spontaneous contractions, I think the contractions he observed after section of the sciatic must have been spontaneous contractions and not due to sympathetic stimulation.

The path of the fibres from the sympathetic to the sciatic can only be by the rami communicantes of the nerves which join the plexus out of which the sciatic arises. Including the variations in different frogs, these are the 7th, 8th, 9th and 10th nerves. I have already mentioned that the 7th nerve peripherally of its ramus has, usually at any rate, no vaso-motor fibres for the web. In three experiments on the 10th nerve, the plexus varying from median to posterior, the 10th had none either. In a few experiments I stimulated the sympathetic chain at the 7th ganglion before and after cutting the 8th and 9th nerves below their rami; the section completely prevented the stimulation from causing arterial contraction in the web.

The vaso-motor fibres, then, must run from the sympathetic to the sciatic in the 8th or 9th nerves or in both of them.

These nerves I have stimulated peripherally of their rami in 12 experiments. In 10 experiments both 8th and 9th nerves caused more or less good contraction of the arteries. The circulation was generally watched in two adjoining arteries of the 3rd web, and as a rule the stimulation caused complete occlusion of them. The relative effect of the two nerves varied somewhat in different cases, sometimes one and sometimes the other had the greater effect, but I was unable to determine

that the relative effect bore any constant relation to the type of lumbo-sacral plexus; in most cases the plexus was dissected and it varied from anterior to median (*b*)[1] forms. If there were such relation, the 8th nerve should have the greater effect with an anterior plexus, and the 9th with a posterior plexus.

In a few cases the relative effect of the two nerves upon the arteries of the different webs was compared; in one case the effect of the 9th nerve was greater in the inner webs, and that of the 8th greater in the outer webs, but in the other experiments there was no certain difference in this respect, though the effect on different arteries was unequal. I conclude that ordinarily each nerve sends vaso-motor fibres to all the arteries, but that any particular artery may receive more fibres from one than from the other.

In the two remaining experiments, the 9th nerve caused good contraction, the 8th nerve caused slight contraction in one and had a doubtful effect in the other. The lumbo-sacral plexus in the latter was anterior, in the former it was not dissected. I do not feel certain that in these experiments the slight effect of the 8th may not have been due to some fault in the experimental conditions.

The preceding account shows that there is large overlapping in the vaso-motor areas of the 8th and 9th nerves. Since, with considerable variations in the origin of the sciatic, each nerve supplies the whole of the foot, it may fairly be concluded that the overlapping extends some distance along the anterior and posterior borders of the leg.

Observations on the 7th, 8th and 9th nerves outside the vertebral canal have been made by Arkharoff (1886). He found that the 7th nerve generally caused dilation, but sometimes contraction, that the 8th nerve usually caused dilation and the 9th nerve contraction, but that sometimes the 8th nerve caused contraction and the 9th dilation; so that each caused dilation and contraction at different times in the course of the same experiment. Arkharoff does not mention whether he stimulated centrally or peripherally of the rami communicantes; and it appears clear from his account that he was unaware of the importance of this. If he stimulated sometimes centrally and sometimes peripherally of the rami, as he naturally would if he thought it of no consequence, his results are not inconsistent with mine. The dilation of the vessels, about which I shall say more presently, commonly results from stimulation of the 7th nerve past its ramus, and of the 8th and 9th nerves centrally of their rami. Contraction results from stimulation of the 7th centrally of its ramus or of the 8th and 9th past their rami.

[1] Cf. Fig. 1, Langley and Orbeli. This *Journal*, XLI. p. 452. 1910.

Correspondence between sympathetic and sensory areas of web.

I have shown in earlier papers that in the mammal the distribution in the skin of the sympathetic fibres contained in a spinal nerve at its union with a plexus is approximately the same as the distribution of the sensory fibres of the nerve. We have seen above that ordinarily both the 8th and the 9th nerves send fibres to the whole of the foot, and that within considerable limits of variation in the origin of the spinal nerves, the 7th and 10th nerves send none. It has been shown by several observers[1] that ordinarily the 8th and 9th nerves are the only nerves which send sensory fibres to the foot, and that each supplies sensory fibres to the whole of it.

We may conclude then that the relation which holds in the mammal between the distribution of the sympathetic fibres running to a nerve of the limb plexus and of the sensory somatic fibres of the nerve holds also in the frog.

It is true that in Sherrington's[2] observations, the foot of the frog occasionally received sensory fibres from the 7th nerve. But I take it that in these cases the sciatic was more anterior in origin than in any of my experiments and that the foot received vaso-motor fibres as well as sensory from the 7th nerve. It is possible also that extreme forms of posterior origin of the sciatic may occur in which both sensory and vaso-motor fibres run to the foot from the 10th nerve.

Passive dilation of the vessels. Vaso-dilator nerves.

The nerve-roots which send vaso-constrictor fibres to the foot send vaso-constrictor fibres to other parts of the body. Contraction of the arteries in any one region will tend to cause a rise of blood-pressure, and the rise of blood-pressure will tend to cause a quicker circulation and some dilation of the vessels in other regions. As has been noticed by earlier observers, a primary increase in the blood-flow of the vessels of the web is a frequent phenomenon in nerve stimulation. In my experiments the primary increase was more frequent, and was much better seen with a feeble than with a good circulation, a fact which indicates that the increase was passive. The increased rate of circulation is generally more obvious than the dilation of the vessels, though the latter is often plain. I have observed an increase in the circulation

[1] For an account of these observations cf. v. Rynberk, *Ergeb. d. Anat. u. Entwick.* XVIII. 1908.

[2] *Phil. Trans. Roy. Soc.* 184. B. p. 650. 1893.

before arterial contraction begins on stimulating each of the spinal nerves containing vaso-motor fibres. It was greater with the 3rd, 4th and 5th nerves than with the 6th and 7th; and this may naturally be referred to the large visceral area supplied by the former. An increase occurs also in the web of the opposite side of the body to the nerve stimulated; here it is not brief but lasts half a minute to several minutes.

The effect of stimulating the nerves of the limb-plexus past their rami is as follows: the 7th nerve commonly causes an increase of blood-flow and slight dilation of the vessels. The 8th and 9th nerves and the sciatic cause as a rule in fresh irritable preparations no increase or but a trifling one, in less irritable preparations they cause a primary increase and some dilation of the vessels; after repeated stimulation there is no effect or only a slight increase in blood-flow; if the blood-flow has stopped, the primary effect of the first stimulus is a rush of blood through the arteries into the web, there may also be a back flow of blood into the veins. Stimulation on one side causes also an increase of blood-flow in the opposite web; this cannot be due to vaso-dilator fibres, it can only be due to rise of arterial blood-pressure. The effect on the same side is greater than that on the opposite side. This is especially the case after the circulation has stopped, the greater effect is no doubt mainly due to the closer connection of the arterial trunks on the same side, though contraction of the arterial trunks may play some part in it.

It is clear from these facts, as indeed from general physical principles, that stimulation of any nerve containing vaso-motor fibres tends to cause a quicker circulation in all parts of the body outside the affected region. How far this is accompanied by dilation of the vessels in any one area depends upon the tone of the arteries in this area and upon the extent of the rise of blood-pressure. It tends also to cause a quicker circulation and a dilation of vessels in any part of the affected region in which the arteries contract more slowly than in the rest of the region.

The facts are of importance in relation to the question of the existence of vaso-dilator fibres. Both Ellis and Arkharoff consider that vaso-dilator fibres are present in the sciatic. Ellis (1885) found that now and then stimulation of the peripheral end of the sciatic caused dilation of the arteries of the web instead of contraction. The effect was obtained by using weak shocks and shocks of slow rhythm but was not constant, and it was better seen a day or two after section of the sciatic—strong shocks of quick rhythm always caused contraction. I have obtained somewhat similar results a day or two after section of

the sciatic nerve, but the experiments are inconclusive, for, if any arteries outside the web were at the moment of stimulation more irritable than those in it—and this might normally be the case, or result from the unequal time of spontaneous contraction in the two regions, or from partial drying or irritation of the web vessels—passive dilation of the vessels of this web would be obtained. The experiments of Arkharoff have been spoken of above. Here the probability that the dilation was passive is stronger, for he commonly found dilation from the 7th nerve, and the 7th nerve rarely runs to the web.

But whilst we may say that there is no proof of the presence of vaso-dilator fibres in the sciatic, we cannot say that their presence is definitely disproved. They might ordinarily be overpowered by the vaso-constrictors.

Stimulation of posterior roots.

Pflüger (1856) stimulated the posterior roots of the nerves which run to the leg, the roots apparently were not stimulated separately. He observed no change in the vessels of the web.

I have made a few experiments on the posterior roots of the 7th nerve, taking this as the type of the nerves containing sympathetic vaso-motor fibres. No effect of any kind was obtained on the circulation in the web.

In the experiments mentioned above on the question of the presence of vaso-constrictor fibres in the roots of the 8th and 9th nerves, I bore in mind the possible presence of 'antidromic' afferent fibres in the posterior roots capable of causing dilation of the arteries. In one experiment (*R. esc.*) the posterior roots of these nerves had no trace of effect; in another (*R. temp.*) they caused at times some quickening of the blood-flow. In the remaining four experiments, the posterior and anterior roots were stimulated together; in two no effect was obtained; in two there was at times some quickening of the circulation. The results then were not decisive; the quickening, when it occurred, may have been caused by some defect in the experimental conditions, and I propose to make further experiments in the matter.

Some phenomena of the circulation and of the effect of nerve stimulation on it.

I have mentioned above that different arteries respond unequally to nerve stimulation. There is a most marked difference between the arteries of the web and those of the toes. The latter contract less

readily than the former, and it is a common phenomenon, at any rate of declining irritability, to find that stimulation of the sciatic or the sympathetic stops the circulation in the web but not that of the toes. Since there are some anastomoses between the smaller veins, blood from the capillary area of one vein can pass by capillaries to another vein, it may happen that in the part of the web observed under the microscope, the blood-flow on nerve stimulation may stop in the artery and in the capillaries, but continue or even become quicker in the vein; and in an anastomosing vein it may reverse direction. After cessation of arterial flow, blood may still pass out of the capillaries for a time, this is no doubt due to the elastic recoil from their previous distension. As other observers, I have not found any satisfactory evidence that the capillaries contract on nerve stimulation. The only indication of active contraction is that in some cases on protracted nerve stimulation they become completely emptied of blood, instead of, as is usual, remaining partly full; the fact however might be caused by an unusual pressure in the surrounding tissues, either normal or in consequence of the presence of lymph.

As a rule the first part of the artery to close, is that near the capillaries, the contractions spread both ways from this, driving blood on into the capillaries and backward in the artery. But with slow circulation and diminished irritability I have seen the artery contract some distance from its ending, and the contraction pass slowly down it a variable distance. Thus in one case in which the circulation had stopped, stimulation of the roots of the 7th nerve caused contraction of the proximal part of the arteriole and the blood was driven into the peripheral part of the artery; on stimulating the roots of the 6th nerve, the latter part contracted and the blood was forced on into the capillaries.

It was noticed by Humilewski and by Jegorow, that after the contraction of arteries following nerve stimulation, there was for a short time a back-flow of blood in the veins. This I have most frequently noticed after the stimulus has ceased, and when the circulation was not very rapid; it was the first sign of a restoration of the blood-flow, lasting 5 to 10 seconds before the flow in the adjoining artery began. This is no doubt chiefly due to the vein anastomosing with some other, the artery of which had dilated, but it might also be due to slight relaxation of the vein. Whilst the veins do not obviously contract on stimulating the sciatic, they sometimes appear to contract slightly.

When the sciatic or the 8th or 9th nerve after repeated stimulation causes no contraction in the vessels of the web, it may sometimes

be seen that the circulation slows considerably a few seconds after the stimulation has ceased; this is unaccompanied by contraction, and is no doubt due to the dilation in other regions of vessels which have slowly contracted during the stimulation.

The capillary course from artery to vein offers various degrees of resistance and there may be blood-flow in an artery, one or two capillaries and the veins when the blood-flow has ceased in nearly the whole of the capillary area. It is probable that in the mammal there is a similar arrangement and that it is of considerable importance in shock, allowing some blood to pass to an organ when the blood-pressure is very low.

General Summary and Remarks.

The vaso-motor fibres for the hind foot of the frog usually leave the spinal cord in the 3rd to the 7th spinal nerves. Probably with a very anterior arrangement of the nerves, the 7th has no vaso-motor fibres. As a rule the effect of stimulation, which may be taken as showing the number of nerve fibres, increases from above downwards, but occasionally the 6th has a greater effect than the 7th nerve. The origin of sympathetic fibres for the foot is then much more extensive in the frog than in the mammal; in the mammal the highest nerve to send sympathetic fibres to the foot is about the 10th thoracic, i.e. the origin of these fibres is some distance from the origin of the somatic fibres of the brachial plexus. In the frog the origin of the sympathetic fibres for the foot overlaps the origin of the somatic fibres of the brachial plexus.

Each nerve does not necessarily supply the whole of the arteries of the web, the 3rd nerve may have no visible effect on some arteries whilst causing complete closing of others, and even with the 7th nerve the effect is unequal in different arteries, but the same artery may be made to contract by each of the nerves. The arteries of the toes are less affected than those of the web. The different parts of any one artery do not contract with the same ease; the more effective nerves usually first cause contraction in the artery close to its end, and then the contraction spreads in both directions; with less effective nerves, one may cause greater contraction in one part, and another in another part.

The vaso-motor nerves pass by the rami communicantes of the corresponding spinal nerves to the sympathetic chain, they leave this in the rami communicantes of the 8th and 9th spinal nerves, and run

in the sciatic nerve to the periphery. No fibres for the web take the course of the blood-vessels. Thus the course taken by the sympathetic fibres in the frog is similar to that taken by the fibres in the mammal.

Usually at any rate the sympathetic fibres of both the 8th and the 9th nerves supply the whole of the foot; it has been shown by earlier observers that both the 8th and 9th nerves supply sensory fibres to the whole of the foot. Thus there is large overlapping of the skin field of both sympathetic and sensory fibres of the nerves which form the lumbo-sacral plexus. This relation between the peripheral distribution of the sympathetic fibres running to a nerve and that of the sensory fibres of the nerve occurs also in the mammal.

The posterior root of the 7th nerve, in the experiments made, had no vaso-motor fibres, and it may be inferred that ordinarily, sympathetic vaso-motor fibres do not leave the cord by the posterior roots.

Stimulation of any nerve containing vaso-motor fibres tends to cause an increased rate of circulation and some vascular dilation in all parts of the body in which the arteries have not contracted as the result of the stimulus. The arteries affected by any one nerve do not contract with equal ease, consequently the stimulus may cause a primary increase in the rate of circulation simulating the effect of vaso-dilator nerves. No clear evidence was obtained of the existence of vaso-dilator nerve fibres for the web.

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[1] I wish to thank Prof. Nussbaum for his kindness in sending me an account of this Paper, at a time when I was unable to obtain the original.

β-IMINAZOLYLETHYLAMINE A DEPRESSOR CONSTITUENT OF INTESTINAL MUCOSA.

By G. BARGER and H. H. DALE.

(*From the Wellcome Physiological Research Laboratories.*)

The description of a symptom-complex common to the action of commercial "peptone" and of extracts of certain organs, such as intestine, brain, thyroid gland, etc. has formed the subject of numerous papers by Popielski and his co-workers[1]. Lohmann[2] and von Fürth and Schwarz[3] have identified choline as a depressor constituent of such extracts, but, as Popielski and his followers have made clear, the very marked depressant action of these extracts cannot be entirely due to choline, whatever be the final outcome of the discussion as to whether pure choline is depressant. The effects attributed by Popielski to the hypothetical "vaso-dilatin" include fall of blood-pressure, due to vaso-dilatation; loss of coagulability of the blood; violent peristalsis, contraction of the bladder; accelerated secretion of saliva, pancreatic juice and, to a less extent, of bile; depression and narcosis. He regards as fundamental effects the fall of blood-pressure, due to "paralysis of the peripheral vasomotor apparatus," and the abolition of the coagulability of the blood, the other effects being secondary to these. He identifies the "secretin" of Bayliss and Starling with "vaso-dilatin," so that the action of secretin on pancreatic secretion is, according to his view, a secondary and non-specific effect.

Though the repeated association of certain actions is suggestive, it by no means amounts to a proof that they are all produced by a single chemical substance. One of us recently, in conjunction with

[1] Popielski. *Pflüger's Archiv*, CXXVIII. p. 191. 1909. Modrakowski. *Ibid.* CXXXIII. p. 291. 1910.

[2] Ref. *Zentralbl. f. Physiol.* XXII. p. 616. 1908.

[3] *Pflüger's Archiv*, CXXIII. p. 361. 1908.

P. P. Laidlaw[1], described the action of β-iminazolylethylamine and drew attention to the fact that it produced all the effects attributed to "vaso-dilatin" with the exception of that on the coagulability of the blood. Further experiment, of which the details will be published later, has confirmed the absence of effect on coagulability, but has revealed a further point of similarity in that β-iminazolylethylamine increases the flow of lymph from the thoracic duct. Since the purest preparation of "vaso-dilatin" yet obtained by the Lemberg laboratory produced, in a dose of 3·6 mgms., a fall of blood-pressure which, so far as Modrakowski's[2] experiments are comparable with our own, would correspond to a much smaller dose of iminazolylethylamine, it seemed desirable to investigate the possibility that the latter substance might be a constituent of "vaso-dilatin." As in Dale and Laidlaw's paper we shall, for the sake of brevity, refer to the base as β-I.

Having found that all the extracts and preparations in question produce the characteristic action on the non-pregnant uterus of the cat and guinea-pig, we used the response of the latter as a physiological indicator in tracing the active substance. Since intestinal extracts were especially active in producing the vaso-dilator fall of blood-pressure and contraction of the guinea-pig's uterus, we took such an extract for investigation. We were not immediately concerned with the specific effect of secretin on pancreatic secretion, but with the associated depressor action. The highly depressant extract of ox-gut with its comparatively small effect on the flow of pancreatic juice, was, therefore, quite suitable for our purpose.

Small intestines of the ox, fresh from the slaughter-house, were washed through by a stream of water and placed in cold storage at − 2° C. Next day the mucous membrane was scraped off and ground with sand. Enough dilute hydrochloric acid was added to make the concentration of the semi-fluid mass equal to 0·1 % HCl. It was boiled, nearly neutralised, and filtered at the pump. In this way, 750 c.c. of extract were obtained from each kilo of "scrapings."

The extract was concentrated, and 20 % tannin was added to it until the precipitate flocculated. The solution then filtered clear; with excess of tannin this was not so. The filtrate was further treated according to Kutscher's method and the silver precipitate produced by adding excess of baryta in the presence of silver nitrate was collected (precipitates Ag. II and Ag. III). From this the bases were regenerated and extracted with hot absolute alcohol. On evaporation of the alcohol

[1] This *Journal*, XLI. p. 318. 1910. [2] *Loc. cit.*

a syrupy residue remained. This was treated with saturated aqueous solution of picric acid, and yielded 19 milligrams of a picrate which soon crystallised, and then melted at 228° C. On recrystallisation it formed yellow serrated needles and rhombic leaflets, melting at 232° C., which were quite similar to the crystals of β-iminazolylethylamine dipicrate, and like the latter gave an intense colouration with p. diazobenzene sulphonic acid (Pauly's histidine reaction).

The picrate thus corresponded chemically with the dipicrate of β-iminazolylethylamine. For physiological comparison 10 mgms. of the recrystallised picrate were dissolved in dilute HCl and the solution freed from picric acid by repeated shaking with ether. Ether was removed from the watery layer by boiling, and the solution was neutralised and made up to 4 c.c. Assuming that the substance was correctly identified each c.c. would then contain approximately 0·5 mgm. of the base as hydrochloride. This is called solution *A*. For comparison a 0·05 % solution of β-I. hydrochloride, obtained from histidine, was used, and is called solution *B*.

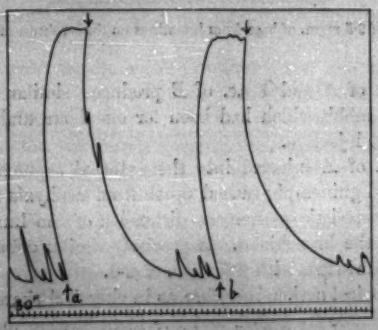

Fig. 1. For description see text. Upstroke = contraction. Fresh Ringer at ↓ ↓.

The following experiments were made:

(1) An isolated horn of the uterus of a virgin guinea-pig was suspended in a bath containing 250 c.c. of oxygenated Ringer's solution at 38° C. Addition to the bath of (*a*) 0·1 c.c. of solution *A* (= 0·05 mgm. of base from intestines); (*b*) 0·1 c.c. of solution *B* (= 0·05 mgm. of β-I.) produced very similar contractions of the uterus (Fig. 1). Solution *A*, presumably containing 0·1 % of base, was as

might be expected, a little more active than *B*, which contained 0·1 % of hydrochloride.

(2) 1 c.c. of solution *A*, injected intravenously into a cat under ether, caused a fall of arterial blood-pressure and increase in volume of a fore-limb (Fig. 2), identical with that produced by the same dose of *B*.

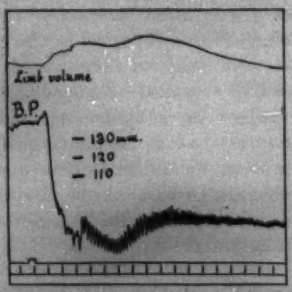

Fig. 2. Effect of 0·5 mgm. of base from intestines on limb-volume and blood-pressure.

(3) 1 c.c. of *A* and 1 c.c. of *B* produced similar rises of blood-pressure in a rabbit which had been for one hour under urethane at the time of the injection.

(4) 1 c.c. of *A* injected into the external saphenous vein of an unanæsthetised guinea-pig caused death from asphyxia in six minutes, with the characteristic permanent distension of the lungs, due to firm contraction of the bronchioles. A precisely similar effect was obtained by Dale and Laidlaw with 0·5 mgm. of β-I.

In physiological action, therefore, as in chemical characters, the base is indistinguishable from β-I., and the identification may be regarded as complete. The yield of picrate which we obtained corresponds only to a small fraction of the activity of the original extract on the blood-pressure or the isolated uterus. The losses, however, due to the unmanageable nature of the bulky precipitates produced, are necessarily very great, and the small yield cannot be regarded as disproving the possibility that β-I. is responsible for practically the whole of the activity in these directions of the original extract. On the other hand it is possible that other substances of somewhat similar action are present.

The hypothetical vaso-dilatin must, therefore, be regarded as consisting of at least two substances:

(1) β-iminazolylethylamine, causing fall of blood-pressure, and the other characteristic effects on plain-muscle and gland-cells, but not affecting coagulation of the blood.

(2) Another substance, or other substances, which render the blood incoagulable and which may or may not play some part in the other effects.

We have no evidence with regard to the origin of the β-I. in the extract of intestinal mucosa. All possible precautions were taken to avoid putrefaction before the material was worked up: moreover a piece of intestine removed immediately after death, or even during life from an anæsthetised animal, washed, scraped and worked up immediately gives an extract with the characteristic physiological action of β-I. Bayliss and Starling[1] showed that the "depressor substance" could be extracted from fresh mucous membrane of dog's intestine by alcohol. It must probably, then, be regarded as a normal product of intestinal mucosa, though whether it is present in living cells, or only formed when these are killed and disintegrated, remains uncertain.

We have made no chemical examination of the similarly acting extracts of other organs, but it seems highly probable that the same base would be found in them. We may mention, in this connection, that we have found the widely varying vaso-dilator action of different samples of "peptone" to run parallel with their activity on the isolated uterus.

It may be desirable to emphasise the fact, already dealt with by Dale and Laidlaw, that this constituent, at least, of "vaso-dilatin," although producing a small secretion of pancreatic juice, has an action in this direction of an altogether lower order than that of an equally depressant dose of secretin. On the other hand, the relatively very weak action of extracts of the large intestine or of "peptone" on pancreatic secretion may probably be adequately accounted for by their content of β-iminazolylethylamine. The effect of the latter on pancreatic secretion is readily abolished by atropine.

Summary.

Popielski's hypothetical "vaso-dilatin" contains β-iminazolylethylamine, which base, however, does not affect the coagulability of the blood.

[1] This *Journal*, xxviii. p. 335. 1902.

ERRATA. VOL. XLI

p. 2. Paper by H. H. Dale, P. P. Laidlaw and C. T. Symons
line 12 from top of page:—
for "1·5 c.c. paraldehyde" *read* "1·5 c.c. paraldehyde per kilo"

p. 156. Paper by J. Barcroft and H. Straub
line 12 from bottom of page:—
for "Albumen Absent" *read* "Albumen Present"

pp. 263—265. Paper by F. W. Edridge-Green
for "Loewi" *read* "Loewe"

CAMBRIDGE: PRINTED BY JOHN CLAY, M.A. AT THE UNIVERSITY PRESS.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

July 9, 1910.

The lipoids of the brain. Part II. A new method for the preparation of the galactosides and of sphingomyelin. By OTTO ROSENHEIM and M. CHRISTINE TEBB.

(*Preliminary communication.*)

In further elaborating the method of selective extraction of brain-lipoids (see this *Journ.* Vol. XXXVIII. p. li., 1909) we have succeeded in finding in pyridine a solvent which makes it possible to extract separately in a state of relative purity both the phosphorus-free galactosides and the saturated crystalline phosphatide sphingomyelin. The advantage of pyridine as a solvent is due to the fact, which we observed previously in connection with the "protagon" mixture, that the galactosides are easily soluble in *cold* pyridine, whilst sphingomyelin is only soluble in *warm* pyridine (at 30°—40° C.).

In applying this observation to the preparation of the lipoids directly from the brain, the plan indicated previously (*loc. cit.*) was followed: cholesterin, water and extractive substances are completely removed by means of cold acetone, a solvent in which the galactosides and practically all the phosphatides (lecithin, kephalin as well as sphingomyelin) are insoluble. The insolubility of sphingomyelin and the galactosides in ether and petroleum ether suggested a further preliminary treatment with one of these fluids, which are well known solvents of the unsaturated phosphatides lecithin and kephalin. The brain tissue is now easily reduced to a fine powder by passing it through a suitable mill and is ready for the treatment with pyridine.

(1) *The preparation of the galactosides.* To ensure thorough extraction with cold pyridine, the powder is first warmed with three volumes of pyridine to 30°—40° C. for a short time (10 minutes) and then cooled down to 15° C. before filtration. A clear reddish extract is obtained, showing well-defined absorption bands

similar to those of hæmochromogen. The extract, which on keeping gradually assumes an olive-green colour, is poured into 3–4 volumes of acetone and a bulky precipitate of impure galactosides is thus obtained. The crude product contains only about 0·5 % of phosphorus and can be separated into its two main constituents, the galactosides *phrenosin* and *kerasin*, by fractional crystallisation from 85 % alcohol at 36° and 0° C. The last traces of phosphorus can be removed by treatment with lead acetate and cadmium chloride (Thudichum), or with zinc ammonium acetate (Thierfelder), or by recrystallisation from glacial acetic acid (Koch).

(2) *The preparation of sphingomyelin.* The extraction is now continued with warm pyridine (3 volumes) at 40°—45° C. for half an hour. The warm clear filtrate is cooled on ice to room-temperature (15° C.). The solution becomes greenish opalescent and a transparent voluminous precipitate settles, which is obtained as a white non-hygroscopic powder after filtration and washing with acetone. The crude product contains already as much as 3 % phosphorus and from it sphingomyelin may be prepared by the process outlined in a previous communication (*loc. cit.*).

A very delicate colour test which served us as a proof for the presence or absence of galactosides in sphingomyelin and other phosphatides, may be mentioned here. The test is carried out by boiling a few milligrammes of the substance with water and adding two drops of an alcoholic solution of *a*-naphthol (Molisch's reagent) to the cold suspension. A fine purple ring develops when concentrated sulphuric acid is allowed to run below the mixture.

It will be seen that in the method outlined above the brain tissue is not exposed for any length of time to the highest temperature employed (45° C.) and any hypothetical chemical or physical changes of the lipoids are thus excluded as far as possible. The method of selective extraction as applied to the brain has so far led to the separation of the following lipoids:

(1) Cholesterin (by extraction with cold acetone).

(2) Lecithin and Kephalin (by extraction with ether or petroleum ether).

(3) Phrenosin and Kerasin (by extraction with cold pyridine).

(4) Sphingomyelin (by extraction with warm pyridine).

The preparation of these latter lipoids directly from brain forms a striking confirmation of our previous statements, according to which the so-called "Protagon" is not only a mixture, but further does not exist preformed in brain (see *Bioch. Ztschr.* Vol. xxv. p. 151, 1910).

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

October 15, 1910.

The relative influence of the heat and chemical impurity of close air. By LEONARD HILL, R. A. ROWLANDS and H. B. WALKER.

(*Preliminary Note.*)

Our experimental chamber is air-tight with cubic content of about 3 metres, and is fitted with an electric stove, three electric fans in the roof, and glass windows.

First type of experiment. Seven students were shut up in the chamber with one of us—in all eight men. No ventilation was allowed. After 44 min. the dry bulb stood at 87° F., the wet bulb 83° F. The CO_2 had risen to 5·26 %. The O_2 had fallen to 15·1 %. The discomfort felt was great, all were wet with sweat, and the skin of all was flushed. The talking and laughing of the occupants had gradually become less and then ceased.

On putting on the electric fans and whirling the air in the chamber the relief was immediate and very great, and this in spite of the temperature of the chamber continuing to rise. On putting off the fans the discomfort returned. The occupants cried out for the fans.

No headache or after effects have followed this type of experiment which has been repeated five times.

Second type of experiment. Two of us have been shut in the chamber, a subject and an observer.

The electric heater is used to raise the chamber to about 85° F. wet bulb.

The subject inhales through a soda lime tin and exhales through a meter; his pulse frequency, pulmonary ventilation and body temperature are recorded. The putting on of the fans gives complete relief to the feelings of discomfort and lowers the pulse frequency.

Having proved this the subject then ceases to use the soda lime tin. A bag containing CO_2, enough to raise the percentage in the chamber to about 2%, is allowed to escape into the chamber, unknown to the subject.

The sudden increase of CO_2 has no influence on the discomfort—it is not sensed, in spite of the increased depth of respiration. The putting on or off of the fans relieves or induces the discomfort as before.

(A grant in aid has been received from the London Hospital Research Fund.)

The influence of alcohol on the power to hold the breath and to work. By J. F. MACKENZIE and LEONARD HILL.

(Preliminary Note.)

We find that the taking of half an ounce of alcohol extends the time which the subject can hold the breath by about a third. It also enables him to lift his weight up on to a stool a great number of times when he holds his breath.

The subject takes a full inspiration, holds his breath, and lifts his body up as many times as he can up to the breaking point, then takes another breath and lifts again, then another breath and so on until he can do no more. He then rests, takes a mixture with or without alcohol and tries again.

The alcohol is disguised according to the prescriptions used by Dr Rivers, and the subject does not know whether he is getting the mixture with alcohol or not.

Compressed air illness I. Solubility of compressed air in water and oil. By J. F. TWORT AND LEONARD HILL.

(*Grant from the London Hospital Research Fund.*)

50 c.c. of water were shaken for 30 min. at +90 lbs. at room temperature (11·5—15° C.), the pressure was lowered to +20 lbs. in 10—12 min. and some of the contents of the chamber drawn off (1) at once, (2) after a period of rest, (3) after a period of gentle oscillation. The dissolved nitrogen was estimated by the Leonard Hill gas pump, the junctions and tap of which were surrounded with mercury cups.

By keeping the mercury in the pump clean and dry excellent nitrogen results were obtained.

Water shaken for 30′ *at* +90 *lbs.*

Dissolved Air found	Dissolved Air at +90 lbs. Calculated	Dissolved Air at +20 lbs. Calculated	When collected
15·1	15·71	—	at +90 lbs.
14·9	15·28	—	at +90 lbs.
14·42	15·88	5·26	at +20 lbs.
9·26	15·28	5·06	after 2 hours at +20 lbs.
9·78	15·74	5·16	after 2 hours at +20 lbs.
8·26	16·35	5·41	after oscillation for 15′ at +20 lbs.
10·47	15·73	5·21	after oscillation for 5′ at +20 lbs.
6·07	16·19	5·36	after oscillation for 30′ at 20 lbs.

The time to suck off the sample occupied 1—3 min. The pressure fell to +12—13 lbs. during this operation. The solubility was calculated on the basis of Bohr's figures.

No visible bubbles appear on decompressing to +20 lbs. nor at +20 lbs. during gentle oscillation. They appear on decompressing to +0. Clouds of small bubbles appear on roughly shaking at +20 lbs. As far as pertains to the watery part of the body it seems safe to decompress from +90 to +20 lbs. in 10 min. and to pause then long enough to allow the excess of nitrogen to escape from the body. A pressure of +20 lbs. prevents bubbles forming.

Vernon found nitrogen and oxygen were more than five times as soluble in fat as in water at one atmosphere. We have tested this at high pressures.

50 c.c. olive oil (sp. gr. ·917) were shaken at +95 lbs. for 38 min. and allowed to stand at +95 lbs. for 2½ hours. Room temp. 17·3° C. The gases were then extracted from a sample.

Vol. of gases (N. T. P)	Calculated on Vernon's results
N. 35·55	39·21
O. 18	17
CO_2 1·62	1·491

Time spent in collecting sample 1 min. Pressure fell 2½ lbs. during collection. The rest of the oil was left for three days at +92 lbs. falling to +84·5 lbs. The sample gave

Vol. of gases (N. T. P.)	Calculated on Vernon's results
N. 33·61	35·53
O. 15·56	15·4
CO_2 1·55	1·35

The oil gave a slow evolution of visible bubbles after reduction of the pressure in 10 min. from +90 to +20 lbs. even without any oscillation of the chamber. The results give further proof that fat men should be excluded from caisson work. The varying percentage of fat in the blood, chyle, liver, must be an important factor in the evolution of bubbles in the blood of compressed air workers. The less fat in the food eaten in the caisson so much the better.

Compressed air illness II. The desaturation of the arterial blood as measured by the nitrogen dissolved in the urine. By LEONARD HILL, J. F. TWORT and H. B. WALKER.

(*Grant from the London Hospital Research Fund.*)

The subject was compressed to +45 lbs., and remained at that pressure for one hour. He drank a quart of water half-way through this period. Samples of urine were collected and sealed up every seven minutes, the first at +45 lbs., the remainder during decompression. The bladder was emptied every time.

Type of six experiments all of which show the same kind of result:

H. B. W. 24 years, 10 stone.

Time	Pressure in lbs.	% N. in urine	% N. calculated
4.0	+45	3·93	3·84
4.6 to 4.12	Decompressed to +13	—	—
4.13	+13	3·7	1·79
4.22	+13	2·62	1·79
4.28	+13	2·30	1·79
4.34	+13	1·87	1·79
4.43	+13	1·80	1·79
4.45 to 4.52	Decompressed to +0	—	—
4.55	+0	1·64	0·96
5.0	+0	1·28	0·96

The results show that the nitrogen does not get into equilibrium with the atmospheric pressure under 10—15 min.

As there is free diuresis and 100—150 c.c. may be passed on each collection, it follows that the nitrogen must come from the arterial blood, and that the arterial blood does not get into equilibrium during its passage through the lung.

The stage decompression tables of Boycott, Damant and Haldane adopted by the Admiralty are based on the supposition that the blood does get into equilibrium.

We controvert these tables. We demonstrate the effect of breathing oxygen during decompression by the following type of experiment. Oxygen helps to clear the nitrogen out.

H. B. W.

Time	Pressure in lbs.	% N. in urine	% N. calculated
12.50	+45	3·83	3·84
12.53 to 12.57	Decompression to +30	—	—
12.57 to 1.1	Decompression to +15 Breathed oxygen for 2 min.	—	—
1.2	+15	3·22	1·92
1.4 to 1.7	Breathed oxygen	—	—
1.9	+15	2.13	1.92
1.10 to 1.15	Decompression to +10 Breathed oxygen for 3 min.[1]	—	—
1.16	+10	1·63	1·6
1.23	+10	1·45	1·6
1.30	+10	1·76	1·6
1.32 to 1.39	Decompression to +5	—	—
1.37	+5	1·61	1·28
1.40	Decompression to +0	—	—
1·44	+0	1·35	0·96

[1] Oxygen supply then gave out.

Effect of breathing oxygen on the nitrogen and oxygen dissolved in the urine. By LEONARD HILL, J. F. TWORT, H. B. WALKER, and R. A. ROWLANDS.

(Preliminary Note.)

The subject drinks a quart of water, and after half-an-hour, when diuresis is established, he breathes oxygen and passes water every seven minutes or so. The bladder is emptied each time. The urine is collected in a urine flask and a sample quickly sealed up in a sample bulb, and the gases are extracted with the gas pump. The final effect of the prolonged deep breathing was such that one subject could not use his hands to collect the sample.

SUBJECT I.

N.	O.	
0·976	0·054	Normal.
1·095	0·287	Oxygen breathed.
0·759	0·243	,, ,,
0·670	0·071	,, ,,
0·677	0·041	,, ,,

SUBJECT II.

N.	O.	
1·121	0·014	Normal.
0·914	0·006	Oxygen breathed.
0·670	0·019	,, ,,

SUBJECT III.

1·234	0·034	Normal.
1·289	0·00	Oxygen breathed.
0·964	0·038	,, ,,
0·837	0·483	,, ,,

SUBJECT IV.

1·068	0·031	Normal.
0·924	0·015	Oxygen breathed.
0·825	0·051	,, ,,
0·919	0·357	,, ,,
0·839	0·216	After ceasing to breathe.

The results show that some of the dissolved nitrogen can be got out of the body by breathing oxygen, but not so much as was expected.

They also show that the renal epithelium checks the entry of oxygen into the urine.

The nitrogen results show that the variations in the oxygen results cannot be explained by a leak of the pump. They may be due to varying renal activity.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

November 19, 1910.

The pacemaker of the mammalian heart as ascertained by electrocardiographic curves.

By THOMAS LEWIS[1].

(*University College Hospital Medical School.*)

In a paper published in the July number of *Heart*, an account was given of certain observations on the dog's heart. Electrocardiograms were taken with the string galvanometer from the upper and left lower extremities, and the auricular portions of the normal curves were compared with the auricular portions of curves accompanying beats of the heart excited from various areas of the auricular musculature. It is known that the course of the contraction wave in muscle controls the type of electric curve yielded by such contraction. The supposition was, therefore, that beats of the heart excited from various portions of the auricular musculature would show differences in the auricular portions of the curves, and that excitation of the area from which normal heart-beats started, would yield curves which were duplicates of the normal curves.

The actual experience, in a series of thirteen experiments, was that duplicate curves are obtainable from an area enclosing the upper half

[1] Working under the tenure of a Beit Memorial Research Fellowship.

or two-thirds of the sulcus terminalis, and a small surrounding area. It is in this area that the sino-auricular node of Keith and Flack lies. A closer examination of the site of the pacemaker by this method was found to be impossible, and observations by another method were undertaken in conjunction with the Doctors Oppenheimer.

We used non-polarisable electrodes of the type described by Gotch, and employed in leading directly from the musculature of the heart. The object of these experiments was the determination of the point on the surface of the right auricle, which becomes primarily electro-negative at the contraction of the auricle. In a series of experiments, it was found that the point of initial electro-negativity could be identified within very narrow limits. It lies immediately in the vicinity of the cavo-appendicular angle, that is to say, at the cephalic extremity of the sulcus terminalis.

A number of points on the exposed surface of the auricle were tested against this point, with uniform results in a series of nine experiments. In seven of the experiments, the points from which leads were taken were marked while the heart lay *in situ* (for subsequent identification) and the tissue of the sulcus terminalis and the portion of the auricle bordering upon it were excised and cut into blocks, each of which surrounded one of the points from which a lead had been taken. The blocks of tissue were cut serially from above downwards, and the relationship of the specialised tissue (sino-auricular node) to the point of primary electro-negativity was ascertained.

It was found in all cases without exception that the point of primary electro-negativity lay immediately over the cephalic end of the sino-auricular node, which in the dog occupies the upper half or two-thirds of the sulcus terminalis.

As a general rule, an enlargement of the node was found at this point, and in the seven hearts examined the tissue of this end appeared to have a more specialised character as compared with that which constituted the remainder of the node.

Thus, the electrical observations lead us to a conclusion which in every way conforms to anatomical findings. There is, at the cephalic end of the sulcus terminalis, a specialised mass of tissue, richly innervated. At the same point, the musculature exhibits certain definite electrical phenomena which lead to the belief that the heart-beat originates in its immediate neighbourhood.

The observations, of which an outline is now given, will be fully reported in the November number of *Heart*, 1910.

The comparative effects of yohimbine, protoveratrine and veratrine upon isolated nerve and upon isolated muscle. By A. D. WALLER, M.D., F.R.S.

In a recent communication to the Society—June 18, 1910—on the relation between the refractory phase and the electrical change in nerve Dr Tait concludes from his experiments on yohimbinised nerve, from those of Waller on protoveratrinised nerve and from a detailed comparison of the literature concerned that the two drugs are pharmacologically identical.

In Tait's view the absolutely refractory phase during action is in all probability katabolic, the relatively refractory phase after action is certainly anabolic, and their electrical counterparts are respectively the negative variation and the positive after-variation.

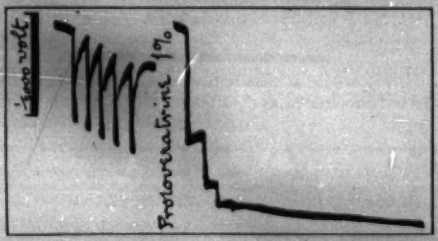

Fig. 1 *a*. Effect of Protoveratrine upon isolated nerve. Negative variations before and after immersion of the nerve for 1 minute in a 1 per 100 solution of Protoveratrine.

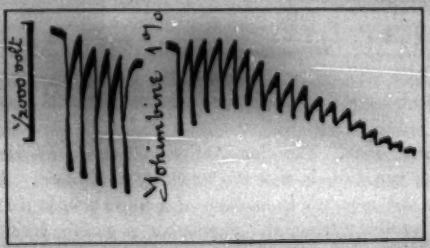

Fig. 1 *b*. Effect of Yohimbine lactate upon isolated nerve. Negative variations before and after immersion of the nerve for 1 minute in a 1 per 100 solution of Yohimbine lactate.

Dr Tait has been good enough to forward to me two samples of yohimbine (the hydrochloride and the lactate) in order that I might make a direct comparison of its effect upon nerve with that of protoveratrine.

Using a fresh $\frac{1}{100}$ solution of yohimbine lactate and a fresh $\frac{1}{100}$ solution of protoveratrine (Boehm) the following results were obtained. Taking as the formula of protoveratrine $C_{32}H_{49}NO_{11}$, and of yohimbine lactate $C_{32}H_{36}N_2O_4 . C_3H_6O_3$, this strength corresponds to a 0·017 normal solution of protoveratrine, and a 0·028 normal solution of yohimbine.

Fig. 2 *a*. Effect of Protoveratrine upon isolated muscle. Sartorius; immersed in 1 per 1000 solution of Protoveratrine as signalled.

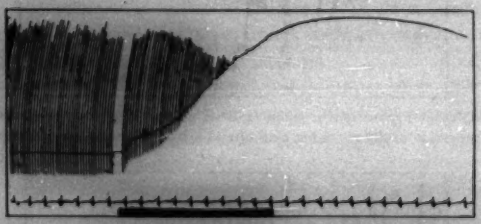

Fig. 2 *b*. Effect of Yohimbine lactate upon isolated muscle. Sartorius; immersed in 1 per 1000 solution of Yohimbine lactate as signalled. (Time in minutes.)

On isolated nerve. The characteristic effect of protoveratrine upon the negative variation is not produced by yohimbine. As shown in Fig. 1, after one minute's immersion of a frog's sciatic nerve in a 1 % solution of protoveratrine, the successive negative variations are of rapidly diminishing magnitude and remarkably prolonged, presenting the characteristic feature of a descending staircase in which successive downward steps are progressively smaller, whereas after immersion for

one minute in a 1% solution of yohimbine the successive negative variations diminish without presenting any sign of permanent after-effect. The declining series of negative variations in the case of yohimbine does not present any sign of the staircase characteristic of protoveratrine (and in less degree of aconitine), and it is considerably less active upon nerve than are either of the two last named alkaloids.

On isolated muscle, with 1 per 1000 solutions of the two alkaloids and by the method demonstrated at a previous meeting of the Society, protoveratrine is less active than yohimbine. At those strengths, as shown in Fig. 2, contractility is abolished in about 15 minutes in the case of protoveratrine, in seven to eight minutes in the case of yohimbine, without contracture in the former case, with marked contracture in the latter. With protoveratrine at twice this strength, viz. at 2 per 1000, twitching occurs resembling that produced by nicotine. With yohimbine at half strength, viz. at 1 per 2000, contractility is abolished without contracture.

Taking into reckoning the molecular weights of the two drugs, their molecular toxicities in relation to muscle are nearly the same, being approximately one half that of quinine and of the same order of magnitude as that of cocaine and of stovaine, viz. between 40 and 50, the toxicity of quinine being taken = 100.

Veratrine (*Cevadine*) as regards its direct action upon immersed muscle is far more active than protoveratrine; in 1 per 1000 solution (0·0017 n.) it causes immediate abolition of contractility and intense contracture, whereas protoveratrine at 1 per 1000 (0·0017 n.) causes abolition of contraction in 15 minutes without any contracture. Veratrine at 1 per 10,000 causes abolition of contraction in 15 minutes without contracture, so that as regards muscle the toxicity of veratrine is to be reckoned as being about ten times that of protoveratrine.

In relation to isolated nerve the degree of toxicity of the two alkaloids is the reverse, protoveratrine being considerably more active than veratrine. Moreover after the subcutaneous injection of protoveratrine an isolated nerve exhibits the characteristic descending staircase, whereas after subcutaneous injection of veratrine an isolated nerve exhibits no appreciable change. The results on muscle taken in conjunction with those previously described on nerve are confirmatory of the conclusion that protoveratrine especially affects nerve while veratrine especially affects muscle. The characteristic effect upon nerve produced by protoveratrine is not produced by yohimbine.

P.S. Dr Tait and I have made observations together of the action of freshly prepared solutions of protoveratrine (Duncan and Flockart, Edinburgh) and of yohimbine lactate (Chemischer Fabrik, Güstrow) comparing on the galvanometer excised sciatic nerves of frogs poisoned with the two drugs respectively. We observed together in the *protoveratrinised* frog the characteristic descending staircase of permanent negative variations as figured above, and in the *yohimbinised* frog an ordinary series of fugitive negative variations, in which the positive after-deflection was particularly evident.

On the latency of sensory nerve endings to mechanical stimulation. By W. A. JOLLY.

(From the Physiological Laboratory, Edinburgh University.)

When the skin of the hind limb in the decapitate cat is stimulated mechanically an electrical variation can be recorded by means of the string galvanometer from the afferent nerve trunk. The arrangement of the experiments performed is as follows:—non-polarisable electrodes are applied to the anterior crural nerve, one to the longitudinal surface and the other to the cut central end. The skin is stimulated by means of a light metal hammer which falls upon a vulcanite plate furnished with sharp points on the lower surface. The upper surface of the plate is covered with metal foil and the contact of the hammer with the foil closes the circuit of an electrical signal. The movements of string and signal are recorded together on a photographic plate, moving at a rate of 850 mm. per sec.

The demarcation current of the nerve is compensated and the negative variation caused by the skin stimulation is recorded.

When a point on the inner side of the thigh is stimulated, a latency of 3·7 σ in the electrical response has been obtained. Deducting 0·8 σ for conduction in the nerve at the rate of 120 meters per second, the latency of the nerve endings in the skin would be 2·9 σ.

On stimulating a point on the inner aspect of the dorsum of the foot, latencies averaging 5 σ have been obtained, which after deduction of conduction time, yield a latency of 2·6 σ.

A similar negative variation can be recorded from the anterior crural nerve when the patellar tendon is struck. The latency of the response has been found to average in 5 preparations 1·5 σ. Deducting 0·5 σ for conduction in nerve, as measured from the middle of the vastus internus to the electrode next the muscle we have an average latency of 1 σ for the nerve endings. The shortest latency observed for the electrical response is 0·8 σ. There is evidence in some of the records that the response is rhythmic.

The effect of the colour of the blood in the blood-vessels of the retina seen subjectively. By F. W. EDRIDGE-GREEN.

If we look through a blue-green glass at a uniformly illuminated white surface as, for instance, a white cloud in the sky, for about 30 seconds, on removing the glass the whole of the field of vision appears rose, with the exception of the centre corresponding to the central non-vascular portion of the retina; this appears bright green.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

December 17, 1910.

The action of trivalent ions on the heart and on certain colloidal systems. By G. R. MINES.

(*Preliminary Note.*)

Eight simple trivalent positive ions which have been examined agree in producing diastolic arrest of the frog's heart when perfused in neutral solution at a concentration of ·00001 M. They also agree in conferring a positive charge on gelatine and on agar-agar and in precipitating in very low concentration a variety of colloidal solutions with negative particles. The complex trivalent positive ion $[Co(NH_3)_6]$ scarcely affects the heart even in concentrations one hundred times greater than the concentration of the simple trivalent ion needed for arrest. This complex ion agrees closely with the simple trivalent positive ions in its power of precipitating a number of negative colloidal solutions (gold sol., arsenic sulphide, boiled diluted egg-white, clay suspension) and in conferring a positive charge on agar-agar: it differs absolutely from them in its inability to precipitate unboiled diluted egg-white or dialysed haemoglobin, or to confer a positive charge on gelatine or on the red corpuscles of the frog.

The trivalent negative ions of the citrate and the phosphate arrest the heart in diastole in concentration ·01 M. They powerfully antagonise

the effects of acid or of trivalent positive ions on the heart. These actions are not shared by the trivalent negative ion of the ferricyanide.

The two former negative ions are powerful in conferring a negative charge on a gelatine membrane, the ferricyanide is inactive in this respect.

In the action of trivalent ions on certain colloidal systems there exists an unexplored factor qualifying the familiar valency law of precipitation and Perrin's law. This factor is of high importance in determining the physiological action of trivalent ions.

On some changes in normal tissues produced by the action of radium. By ALBERT S. GRÜNBAUM AND HELEN G. GRÜNBAUM.

By the application of radium to skin which is subsequently excised, the direct effects of radium on fresh normal human tissue may be studied. A progressive inflammation proportionate to the length of exposure is produced. Relative absence of polymorphonuclear leucocytes and the presence of many plasma cells in the early stages distinguish radium inflammation from chemical or septic inflammation.

The first effects are visible after a few hours; they are perivascular infiltration and the advent of plasma cells. There follow soon leucocytic aggregation in the small vessels together with diapedesis. Around the sweat glands the infiltration is very marked: much less so around the sebaceous glands. After this stage, the exudate contains also many polymorphonuclear leucocytes, which break up and leave a considerable quantity of chromatin debris in the perivascular and surrounding tissue spaces. The epidermis is invaded with leucocytes and many of the basal cells wander or send up processes into the superficial layers. After 24 hours, the epithelium may be raised from the dermis as in a burn or in pemphigus.

If radium be placed in a quartz tube and left for some weeks in the substance of the liver, a capsule is formed around the tube and the liver cells are affected and destroyed within only quite a short distance, with very little remaining interstitial reaction.

In the gall bladder no obvious changes occur.

In the stomach an atrophic fibrosis of the mucous membrane and destruction of the submucosa result.

On incubating eggs in the doses hitherto tried, its effect, like that of X-rays in excess, is to destroy the embryo.

The effect of potassium salts on the circulation, with special reference to the production of heart block. By G. C. MATHISON.

(*Preliminary Communication.*)

While working with decapitated cats it was observed that a few c.c. $\frac{N}{10}$ KOH, when injected into the central end of the stump of the carotid artery, brought about a rise of blood-pressure. This was thought to be due to the alkali action on the wall of the blood vessels, but it was found that equal quantities of $\frac{N}{10}$ KCl or $\frac{N}{10}$ KNO_3 produced almost the same rise.

Experiments shewed that KCl solutions injected into the carotid, so as to avoid their passing first through the heart, produced a rise of blood-pressure; for example 2 c.c. $\frac{N}{2}$ KCl injected over 15 seconds caused the pressure to rise from 92 mm. to 196 mm., 5 c.c. $\frac{N}{10}$ KCl over 20 seconds to rise from 56 mm. to 148 mm., 10 c.c. $\frac{N}{5}$ KCl over 110 seconds to rise from 54 mm. to 180 mm.

This rise is mainly due to vaso-constriction, which is apparently due to direct action on the vessel wall, as it is seen when the spinal cord is pithed. Though many authorities state that potassium salts dilate the blood vessels, it has often been pointed out that this is not the case (see Braun[1]).

When injected into one of the external jugular veins, the salts act first on the heart. With small doses a rise of pressure is seen, with larger doses a fall of pressure with diastolic stoppage of the heart. Occasionally where the heart was directly observed, an acceleration of rate and an increased tone of the muscle were seen when small doses were given. So far I have not observed the fibrillation which Traube[2] and others have described.

In four experiments the production of heart block was noted, both 2:1, and complete block. Recovery took place when the injection was discontinued. Heart block has been observed in other cases in the dying heart, but these are not considered here. It is difficult to administer the right amount to produce heart block, as diastolic standstill frequently comes on at once.

The effects on the heart are at present being more fully investigated, especially with regard to the action of Ca salts and of atropine.

Examples of an experiment in which heart block was produced, and of the effect of the salts on blood-pressure are given below.

Cat, 3·4 kilos. Decapitated, curarised. Injection of 10 c.c. $\frac{N}{5}$ KCl in 3 mins. 10 secs. into external jugular vein.

Mins.	Secs.	Art. pressure in mm. Hg.	
0	0	57	Commenced injection.
1	30	72	Dropped beats.
2	30	74	Continuous heart block.
2	40	66	Injection of 10 c.c. $\frac{N}{5}$ KCl completed.
3	10	108	Continuous heart block ended. Injection restarted.
3	40	112	Still dropped beats.
3	55	64	Continuous heart block. Injection of 2 c.c. $\frac{N}{5}$ KCl completed.
4	30	128	Heart block ended.

Effect of KCl on blood-pressure.

Cat, 3 kilos. Decapitated, curarised.

Injection	Time over which injection was given	B.-P. at start mm. Hg.	B.-P. after injection mm. Hg.
5 c.c. $\frac{N}{8}$ KCl in vein	35 secs.	76	64
3·5 c.c. $\frac{N}{8}$ KCl in artery	25	76	108

Cat, 3·4 kilos. Decapitated, curarised.

3 c.c. $\frac{N}{9}$ in vein	30	57	62
3 c.c. $\frac{N}{9}$ in artery	25	54	80
4 c.c. $\frac{N}{5}$ in vein	40	44	54
3 c.c. $\frac{N}{5}$ in artery	30	44	100
4 c.c. $\frac{N}{5}$ in vein	10	Diastolic standstill of heart.	
5 c.c. $\frac{N}{10}$ in artery	20	56	148

REFERENCES.

(1) Braun. Pflüger's Archiv. cm. p. 476. 1904.
(2) Traube. Ges. Beiträge, Bd. I. S. 883.